THIS NUMBER COMPLETES VOLUME XXXII

VOLUME XXXII DECEMBER, 1953 NUMBER 12

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

Contents

The Prevention of ACTH-Induced Sodium Retention by the Use of Potassium Salts: A Quantitative Study. GRANT W. LIDDLE, LESLIE L. BENNETT, AND PETER H. FORSHAM 1197

Comparative Studies of Serum Polysaccharides in Rheumatoid Arthritis and Degenerative Joint Disease. M. R. SHETLAR, R. W. PAYNE, JANE A. BULLOCK, D. R. PATRICK, ARTHUR A. HELLBAUM, AND WILLIAM K. ISHMAEL 1208

Fructose in the Treatment of Diabetic Ketosis. J. H. DARRAGH, R. A. WOMERSLEY, AND W. H. MERONEY 1214

A Quantitative Study of the Digitoxin Content of Edema Fluids. SHIRLEY ST. GEORGE, CHARLES F. NAEGELE, FRANK S. FRENCH, RAY H. ROSENMAN, AND MEYER FRIEDMAN 1222

Iron Absorption in Dogs During Anemia Due to Acetylphenylhydrazine. W. B. STEWART, PHILIP S. VASSAR, AND ROBERT S. STONE 1225

Studies on Thalassemia. Part I. An Extracorpuscular Defect in Thalassemia Major. Part II. The Effects of Splenectomy in Thalassemia Major with an Associated Acquired Hemolytic Anemia. HERBERT C. LICHTMAN, R. JANET WATSON, FELIX FELDMAN, VICTOR GINSBERG, AND JEAN ROBINSON 1229

Adrenal Cortical Regulation of the Distribution of Water and Electrolytes in the Human Body. A. G. HILLS, T. M. CHALMERS, G. D. WEBSTER, JR., AND OTTO ROSENTHAL 1236

Erythrocyte Metabolism. The Metabolic Behavior of the Cholesterol of Human Erythrocytes. IRVING M. LONDON AND HAIYIM SCHWARZ 1248

Studies on the Composition of the Fibrinoid Material of the Subcutaneous Nodule of Rheumatoid Arthritis. MORRIS ZIFF, THOMAS KANTOR, EDWARD BIEN, AND ALFRED SMITH 1253

The Use of Radioactive Chromium 51 as an Erythrocyte Tagging Agent for the Determination of Red Cell Survival in Vivo. FRANKLIN G. EBAUGH, JR., CHARLES P. EMERSON, AND JOSEPH F. ROSS 1260

Some Clinical, Biochemical and Genetic Observations on Hemoglobin C. HELEN M. RANNEY, DANIEL L. LARSON, AND GEORGE H. MCCORMACK, JR. 1277

The Hemodynamic Effects of Hypotensive Drugs in Man. III. Hexamethonium. EDWARD D. FREIS, JOHN C. ROSE, EDWARD A. PARTENOPE, THOMAS F. HIGGINS, ROBERT T. KELLEY, HAROLD W. SCHNAPER, AND ROBERT L. JOHNSON 1285

The Direct Measurement of the Extracellular Phase of Tissues. GEORGE NICHOLS, JR., NANCY NICHOLS, WILLIAM B. WEIL, AND WILLIAM M. WALLACE 1299

The Body/Venous Hematocrit Ratio: Its Constancy over a Wide Hematocrit Range. H. CHAPLIN, JR., P. L. MOLLISON, AND H. VETTER 1309

Bacterial Digestion of Collagen. JOHN D. MACLENNAN, INES MANDL, AND EDWARD L. HOWES 1317

Isolation and Characterization of Proteinase and Collagenase from Cl. Histolyticum. INES MANDL, JOHN D. MACLENNAN, AND EDWARD L. HOWES 1323

Author Index to Volume XXXII 1330

Subject Index to Volume XXXII 1348

The Journal of Clinical Investigation

Printed by the Lancaster Press, Inc., Prince and Lemon Sts., Lancaster, Pa. Published by the American Society for Clinical Investigation. Publication Office, 622 West 168th St., New York 32, N. Y.

STANLEY E. BRADLEY, EDITOR-IN-CHIEF

ASSOCIATE EDITORS

LUDWIG W. EICHNA IRVING M. LONDON

The Editorial Committee of the American Society for Clinical Investigation

S. HOWARD ARMSTRONG, JR., *Chicago, Ill.*
ROBERT W. BERLINER, *Bethesda, Md.*
WILLIAM J. DARBY, *Nashville, Tenn.*
DAVID P. EARLE, JR., *New York, N. Y.*
RICHARD V. EBERT, *Minneapolis, Minn.*
EUGENE B. FERRIS, JR., *Atlanta, Ga.*
JACOB E. FINESINGER, *Baltimore, Md.*
WARD S. FOWLER, *Rochester, Minn.*
THOMAS HALE HAM, *Cleveland, O.*
FRANZ J. INGELFINGER, *Boston, Mass.*
F. RAYMOND KEATING, JR., *Rochester, Minn.*
JOHN A. LUETSCHER, JR., *San Francisco, Calif*
JOHN P. PETERS, *New Haven, Conn.*
CHARLES H. RAMMELKAMP, *Cleveland, O.*
EDWARD C. REIFENSTEIN, JR., *Oklahoma City, Okla.*

THE JOURNAL OF CLINICAL INVESTIGATION is designed for the publication of original investigations dealing with or bearing on the problems of disease in man, and it is the policy of the editors that the JOURNAL should cover the field of clinical investigation in its broadest sense.

Contributions should be sent to the **Editor-in-Chief, Dr. Stanley E. Bradley, 622 West 168th St., New York 32, N. Y.** In order to hasten the process of reviewing and editing it is requested that each original manuscript be accompanied by a duplicate copy.

In order further to expedite the earliest possible publication of articles submitted to the JOURNAL OF CLINICAL INVESTIGATION they will be published in the order of final processing after review, correcting and editing rather than in the strict sequence of their receipt in this office. Papers which require much editorial change, revision of charts and tables, condensation and rewriting will be delayed according to the time required for their correction. This policy will put a premium on excellence of manuscript as well as the major consideration of content. Because of the increasing costs of printing, especially in the hand work of making tables, the editors suggest that when feasible photographs of tables be prepared. It is also requested that charts be photographed, the photographs accompanying the manuscript. The originals of the charts need not be forwarded except by special request of the editors. Further information regarding the preparation of manuscripts may be obtained from the editors.

Each volume will consist of 12 monthly issues appearing throughout the calendar year. The subscription price is $12 per volume, **payable in advance;** foreign countries also $12, payable in U. S. funds. Single copies will be sold when available at Vols. 1–28, $2; Vols. 29–31, $1.50 a copy. Authors may purchase reprints at scheduled prices. **A special subscription rate of $6 per volume of 12 monthly issues is offered to medical students, interns, fellows, and residents of approved hospitals.** Subscriptions are **net postpaid.** Remittance should be made by draft, check or postal money order, payable to **The Journal of Clinical Investigation, 622 West 168th St., New York 32, N. Y.** Claims for copies lost in the mails must be received within **thirty** days (domestic), **ninety** days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

Acceptance for mailing at special rate of postage provided for in paragraph (d-2), section 34.40, P.L. & R. of 1948, authorized February 13, 1950

Entered as second-class matter September 16, 1931, at the Post Office at Lancaster, Pa., under the Act of March 3, 1879

Copyright, 1953, American Society for Clinical Investigation

CLINICAL INVESTIGATION OF SULFONAMIDE PROPERTIES

Relation to Infections of the Urinary Tract

Nature of the Problem. Among bacterial infections, only those of the respiratory system outnumber infections of the urinary tract. "Simple pyelitis" apparently occurs very seldom. "Infection of the interstitial tissue of the kidney is almost invariably associated with that of the kidney pelvis."[1] It is considered that the infection is often blood-borne.[1]

Chemotherapeutic Approach. As with other infections of parenchyma, the therapeutic agent of choice is one which (a) establishes a high, prolonged blood level; (b) exhibits superior tissue distribution; and (c) has potent antibacterial action.

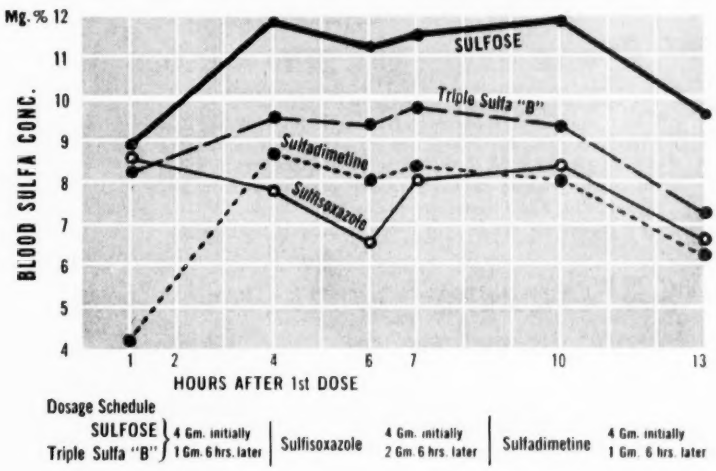
The Safety Factor. In acid urines (pH of 6.0 or less), commonly encountered when infection is present, the danger of crystalluria is less when SULFOSE is given than when other sulfonamides are used. In acid urines, triple sulfonamides are far more soluble than single sulfonamides.

POTENCY AND SAFETY
SUGGEST THERAPY
WITH

S U L F O S E ®

Triple Sulfonamides Wyeth

BLOOD SULFA LEVELS FOLLOWING ORAL ADMINISTRATION OF VARIOUS COMPOUNDS



SULFOSE produces higher and better-sustained blood levels, as shown by comparative clinical studies.[2]

SULFOSE contains sulfonamides noted for superior tissue distribution.[3]

The SULFOSE components show superior,[4] and additive[5] antibacterial action against E. coli.

Supplied: Suspension; bottles of 1 pint
Tablets, 0.5 Gm.; bottles of 100 and 1000

One teaspoonful Suspension (5 cc.) or one Tablet supplies 0.167 Gm. each of sulfadiazine, sulfamerazine and sulfamethazine.

1. Jeghers, H.: GP 6:73 (Sept.) 1952
2. Berkowitz, D.: Antibiotics & Chemotherapy 3:618 (June) 1953
3. Sophian, L.H.; Piper, D.L., and Schneller, G.H.: The Sulfapyrimidines. New York, Press of A. Colish, 1952; p. 56
4. Schnitzer, R.J., et al.: J. Pharmacol. & Exper. Therap. 85:47 (Sept.) 1946
5. Schneller, G.H.: personal communication

Wyeth ®
Philadelphia 2, Pa

Permapen®

Brand of dibenzylethylenediamine dipenicillin G

DBED

PENICILLIN

easy-to-give
easy-to-take
formulations

Pfizer

easy-to-give Permapen AQUEOUS SUSPENSION

As prophylactic therapy in rheumatic fever or in the adjunctive treatment of penicillin-sensitive infections, one intramuscular injection produces blood levels lasting as long as 14 days. *Easy-to-give* Permapen Aqueous Suspension is supplied in single-dose, disposable STERAJECT® cartridges containing 600,000 units of DBED penicillin. Each cartridge comes with sterile, individually wrapped needle, ready for immediate use in your Pfizer STERAJECT syringe.

long-lasting Permapen FORTIFIED AQUEOUS SUSPENSION

Embodies the higher blood levels produced by 300,000 units of procaine penicillin G crystalline and the protracted blood levels obtained from 300,000 units of DBED penicillin. Available in single-dose, disposable STERAJECT cartridges, each with its own sterile, individually wrapped needle.

easy-to-take Permapen ORAL SUSPENSION

Therapeutic benefits are independent of meals, and one teaspoonful—300,000 units of DBED penicillin—every 8 hours provides demonstrable blood levels 'round the clock in most patients. *Easy-to-take* Permapen Oral Suspension is peach-flavored, leaves no disagreeable after-taste. Supplied in 2 oz. bottles. No refrigeration needed.

Pfizer

PFIZER LABORATORIES, Brooklyn 6, N.Y.
Division, Chas. Pfizer & Co., Inc.

Doctor, would it be helpful to you in your practice to know that there is a food available at reasonable prices in the stores the year round having these attributes:

1. One of the best "protective" foods with a well-rounded supply of vitamins and minerals.

2. Low sodium—very little fat—no cholesterol.

3. One of the first solid foods fed babies.

4. Useful in bland and low-residue diets.

5. Mildly laxative.

6. May be used in the management of both diarrhea and constipation.

7. Can be used in reducing diets.

8. Can be used in high-calorie diets.

9. Useful in the dietary management of celiac disease.

10. Useful in the dietary management of idiopathic non-tropical sprue.

11. Useful in the management of diabetic diets.

12. Valuable in many allergy diets.

13. A protein sparer.

14. Favorably influences mineral retention.

15. Useful in the management of ulcer diets.

FOR THE NAME OF THIS FOOD, PLEASE TURN THE PAGE

The answer is

B A N A N A S

If you would like

1. The authority for any of the statements made on the preceding page . . .
2. Additional information in connection with any of them . . .
3. The composition of the banana . . .
4. The nutritional story of the banana . . .
5. Information on various ways to prepare or serve bananas.

Please feel free to write to

Director, Chemical and Nutrition Research
United Fruit Company
PIER 3, NORTH RIVER, NEW YORK 6, N. Y.

READY SOON!—NEW VOL. 6

"Methods in Medical Research"

Edited by J. MURRAY STEELE, M. D.
Professor of Medicine, New York University College of Medicine

CONTENTS AND ASSOCIATE EDITORS

SOME METHODS OF STUDYING HUMAN GENETICS
Antonio Ciocco, Sc.D., *Professor and Head of Dept. of Biostatistics, Graduate School of Public Health, University of Pittsburgh.*

METHODS IN ENVIRONMENTAL MEDICAL RESEARCH
Ray G. Daggs, Ph.D., *Director of Research, Army Medical Research Laboratory, Fort Knox, Ky.*

STATISTICS IN MEDICAL RESEARCH
Donald Mainland, M.B., Ch.B., *Professor and Chairman of Dept. of Medical Statistics, New York University College of Medicine.*

DESIGN AND CONSTRUCTION OF METABOLISM CAGES
Arnold Lazarow, M.D., *Associate Professor, Dept. of Anatomy, Western Reserve University.*

Approx. 280 Pages; Illustrated

Orders may now be placed with your book store or with the publisher. Probable publication date, February 1.

THE YEAR BOOK PUBLISHERS, INC. 200 East Illinois Street, Chicago 11, Illinois

FISHER *Tissuemat*

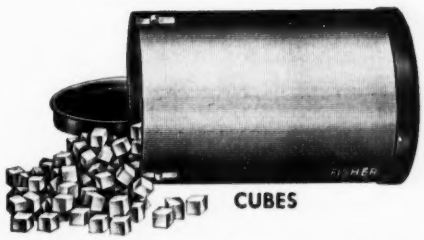
THE SUPERIOR EMBEDDING COMPOUND

Tissuemat is widely used by leading laboratories for impregnating and embedding biological specimens for microscopic examination.

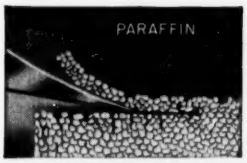
Fisher Tissuemat is superior because:

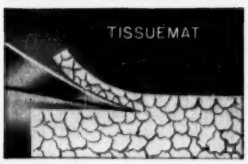
1. It will not crack or crumble during slicing
2. Forms ribbons readily
3. Coheres during slicing
4. Convenient and clean to handle

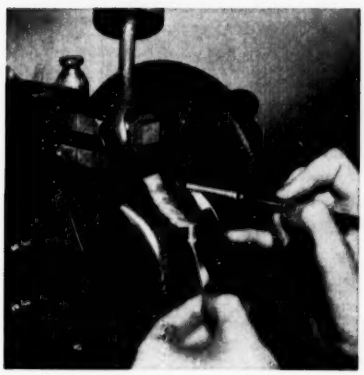
FLAKES



CUBES







The larger cell structure of Tissuemat makes it superior to paraffin because it will not crack or crumble when cut on the Microtome. There is no cell shrinkage or cell distortion during impregnation or cutting.

Tissuemat Flakes are packed in one-pound cartons with moistureproof liners and in ten-pound fiber containers. When ordering, specify melting point range.

1-lb.....$.70 10-lbs.....$6.50

Tissuemat Cubes are about one inch thick, convenient for quick dispensing. The containers are fiber with metal ends.

25-lbs....$13.50 50-lbs....$25.00

Melting point ranges available

A. 50-52°C. (122-125°F.) C. 54-56°C. (130-133°F.)
B. 52-54°C. (125-130°F.) D. 56-58°C. (133-137°F.)
E. 60-62°C. (140-143°F.)

MICROTOMES are available in several models to meet various requirements. Special Microtome knife sharpener also available. Write for complete details.

***FREE*— one pound sample from us or ask your dealer. Specify melting point.**

FISHER SCIENTIFIC

PITTSBURGH (19) 717 Forbes St. | NEW YORK (14) 635 Greenwich St. | ST. LOUIS (18) 2850 S. Jefferson Ave. | WASHINGTON 7722 Woodbury Dr., Silver Spring, Md. | MONTREAL, P.Q. 904 St. James | TORONTO (8), P.O. 245 Carlaw Ave.

America's Largest Manufacturer-Distributor of Laboratory Appliances and Reagent Chemicals

Pro-Sol

STANDARDIZED

Protein Solution

A standardized Protein Solution for Bio-Chemical use that will keep indefinitely.

May be used for calibration of standard protein curves in any colorimetric, photometric or Kjeldahl procedure.

Definite protein content given on each container—determined by the Kjeldahl method.

Package of 4 sterile bottles each containing approximately 3 cc. $7.50 per package.

STANDARD SCIENTIFIC SUPPLY CORP.

34 West 4th Street, New York 12, N. Y.

Serving Science Since 1888

The Journal of Clinical Investigation

622 West 168th Street — New York 32, New York

COMPLETE VOLUMES OF BACK ISSUES

Vols. 1 through 11, inc.—special price	$25.00
Vol. 12 (1933) six issues	10.00
Vol. 13 (1934) six issues	10.00
Vol. 27 (1948) six issues and one supplement	10.00
Vol. 28 (1949) six issues and two supplements	10.00
Vol. 29 (1950) twelve issues	10.00
Vol. 30 (1951) twelve issues and one supplement	12.00
Vol. 31 (1952) twelve issues	12.00

Order While Available

A Christmas Gift Subscription

JOURNAL OF CLINICAL INVESTIGATION
622 West 168th Street, New York 32, N. Y.

Please enter the following name for a one year's subscription, January through December, 1954, Volume 33:

Please Print:

.....

Name

.....

Address City Zone State

Name and address of donor:

.....

.....

Enclosed is $12.00——— Bill me for $12.00———

Special rate of $6.00 a year (pre-paid) for medical students..., interns..., residents..., and fellows.... One of the above *must* be checked if $6.00 is enclosed.

Announcement

an advance in therapy of adrenocortical insufficiency

Percorten® trimethylacetate

DESOXYCORTICOSTERONE TRIMETHYLACETATE CIBA

Intramuscular repository

"A month's therapy with a single injection." Therapy of chronic adrenocortical insufficiency has been improved and made more convenient to both patient and physician by Ciba's introduction of Percorten trimethylacetate in the form of a long-acting microcrystalline suspension.[1-5] • A single injection of this new long-acting steroid hormone derivative provides maintenance therapy for about a month. • This protracted and constant therapeutic effect is achieved because the suspension, when administered by deep intramuscular injection, simulates a miniature pellet in that a small fraction of the total dose is absorbed daily. • Patients who have Addison's disease and who are not in crisis can now be treated on a highly satisfactory ambulatory basis.

Indications: Chronic primary and secondary adrenocortical insufficiency, and hypotension associated with adrenal insufficiency. **Supplied:** Multiple-dose vials—4 ml. containing 25 mg./ml. as an aqueous suspension for intramuscular use only. **Note:** Percorten (desoxycorticosterone Ciba) is available in 4 administration forms: (1) Percorten trimethylacetate, long-acting intramuscular repository, aqueous suspension; (2) Percorten acetate in oil solution, for intramuscular injection; (3) Percorten acetate Linguets,℞ for sublingual or parabuccal absorption; (4) Percorten acetate pellets, for subcutaneous implantation.

Ask your Ciba professional service representative or write the Medical Service Division for complete information on the use of Percorten trimethylacetate.

1. Gross, F. and Tschopp, E.: Experientia *8*:75, 1952. **2.** Thorn, G. W. and Jenkins, D.: In press. **3.** Thorn, G. W.; Jenkins, D.; Arons, W. L., and Frawley, T. F.: Schweiz. med. Wchnschr. *82*:697, 1952. **4.** Gaunt, R.; Leathem, J.; Howell, C., and Antonchak, N.: Endocrinology *50*:521, 1952. **5.** Sorkin, S. Z., and Soffer, L. J.: Am. Fed. Clin. Research, May 4, 1952.

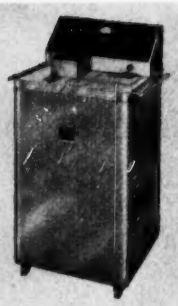
Ciba *Summit, New Jersey*

8/1910M

Time-Saving Versatile Aids for the Laboratory!

ELECTROPHORESIS APPARATUS

Aminco's leadership in this field has made its models of electrophoresis apparatus standard equipment in research institutions throughout the United States and abroad. Compact and complete units, these instruments incorporate optical systems of high resolving power, regulated high-voltage supplies, mechanical refrigeration, and dialysis facilities. Convenient to use, high output, mobile, and versatile for exacting research in electrophoresis, analysis, preparative work, and diffusion studies. BULLETINS L-2175 & L-2281.



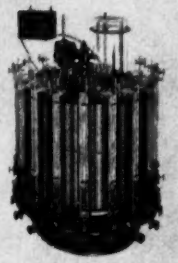
ELECTROPHORESIS APPARATUS

WARBURG APPARATUS

Maximum operating convenience and superlative performance are characteristic of Aminco Warburg apparatus that is serving so creditably the world over.

Available in the rotary type (illustrated) or dual-shaker (rectangular) type, in heated or refrigerated models.

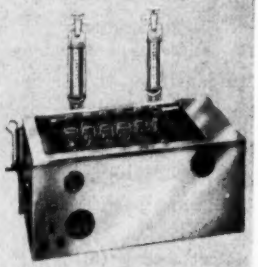
The many advantages of Aminco apparatus are described in BULLETIN L-2185.



WARBURG APPARATUS

DUBNOFF METABOLIC SHAKER-INCUBATOR

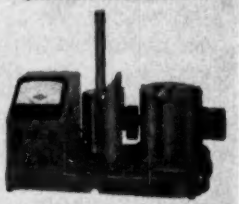
Up to 30 specimens can be shaken simultaneously in this improved apparatus at temperatures from 25° to 100° C., with a sensitivity of plus-minus 0.015° C. within the flasks. Intended for metabolic studies not requiring the measurement of gas exchange. Accommodates two types of racks for holding beakers or Erlenmeyer flasks. Shaking speed is constant regardless of line-voltage fluctuations. Has provisions for gassing. Flasks may be simultaneously cooled, gas equilibrated, incubated, or boiled. Many other features described in BULLETIN L-2239.



SHAKER-INCUBATOR

LIGHT-SCATTERING MICROPHOTOMETER

Measures from 20 micro-microlumens to 20 lumens, absolute turbidity, dissymmetry, depolarization, fluorescence, reflectance, phosphorescence, optical density, and optical transmission. Has outlet for connection to a recorder. BULLETIN L-2241.



LIGHT-SCATTERING MICROPHOTOMETER

PHOTOMULTIPLIER MICROPHOTOMETER

For precise measurement and comparison of light intensities at selected wavelengths. Measures from 20 micro-microlumens to 20 lumens, densities from 0 to 11, and phototube currents from 10^{-5} to 10^{-11} amperes. Range can be extended with neutral filters. BULLETIN L-2190.

PHOTOMULTIPLIER MICROPHOTOMETER

Other Aminco Laboratory Instruments

Freeze-dry Apparatus	Incubators	Thermoregulators
Centrifuges	Ovens	Relays
Pipetting Machines	Sterilizers	Pumps
Optical Absorption Cells	Mechanical Dialyzers	Stirrers
Constant Temperature Baths	Flame Photometers	Immersion Heaters

Coleman Spectrophotometers, Fluorometers, Nephelometers, Colorimeters, and pH Meters

AMINCO *Laboratory Instruments*

AMERICAN INSTRUMENT COMPANY, INC.

Silver Spring, Maryland • In Metropolitan Washington, D. C.

ACKNOWLEDGMENT

The Editors wish to acknowledge with gratitude the generous assistance of the reviewers listed below.

Fuller Albright
Benjamin Alexander
Thomas P. Almy
Alf Sven Alving
D. M. Angevine
W. Parker Anslow
Henry Aranow, Jr.
Samuel P. Asper, Jr.
E. B. Astwood
Robert Austrian
Alvan L. Barach
Fred Barnes
Henry Lewis Barnett
Robert C. Batterman
Walter Bauer
Robert H. Bayley
William B. Bean
Henry K. Beecher
Ivan L. Bennett
Nathaniel Berlin
Robert W. Berliner
Robert Berne
Solomon A. Berson
Richard J. Bing
William D. Blake
Harold F. Blum
Oscar Bodansky
Philip K. Bondy
James Bordley, III
Robert Bowman
Ivan Brown
J. S. L. Browne
J. J. Bunim
George E. Burch
Howard Burchell
Joseph Holland Burchenal
Charles H. Burnett
George E. Cartwright
William B. Castle
I. L. Chaikoff
Alfred Chanutin
K. K. Chen
Francis P. Chinard
Lockard Conley
J. W. Conn
A. C. Corcoran
André Cournand
Jefferson Martineau Crismon
Richard J. Cross
George L. Curran
T. S. Danowski
Robert C. Darling
Daniel C. Darrow
William H. Daughaday
Charles S. Davidson
Bernard D. Davis
Elmer L. DeGowin
Quentin Deming
D. Denny-Brown
Lewis Dexter
Zacharias Dische
Vincent P. Dole
Harry Eagle
Robert H. Ebert
I. S. Edelman
Howard A. Eder
Herman N. Eisen
Leonard P. Eliel
J. Russell Elkinton
Laurence B. Ellis
Charles P. Emerson
Kendall Emerson, Jr.
John F. Enders
Frank L. Engel
Lewis L. Engel
Saul J. Farber
Wallace O. Fenn
Clement Alfred Finch
Stuart C. Finch
Edward Fischel
A. P. Fishman
Richard H. Follis, Jr.
Peter H. Forsham
A. Stone Freedberg
David G. Freiman
Edward D. Freis
Carl K. Friedland
Meyer Friedman
Joseph S. Fruton
Morton Galdston
Alfred Gellhorn
John G. Gibson, II
Alfred Gilman
Gilbert Glasser
Marcel Goldenberg
William Goldring
Leonard Goldwater
Harry H. Gordon
R. Gordon Gould
S. Granick
Seymour J. Gray
Jack Gross
Jerome Gross
Frank R. Gurd
Alexander B. Gutman
Paul F. Hahn
Morton Hamburger
W. F. Hamilton, Jr.
Franklin Hanger
James D. Hardy
Réjane M. Harvey
A. Baird Hastings
Hans H. Hecht
Robert W. Heinle
Albert Heyman
John B. Hickam
S. W. Hoobler
C. Riley Houck
Dwight J. Ingle
Joseph W. Jailer
Harold Jeghers
Robert E. Johnson
Elvin A. Kabat
Eugene Kaplan
Robert M. Kark
David A. Karnofsky
Thomas J. Kennedy
W. Kerson
Albert Keston
Seymour S. Kety
Ancel Keys
Ralph A. Kinsella, Jr.
Irving M. Klotz
Abbie I. Knowlton
Saul Roy Korey
Charles E. Kossmann
Henry G. Kunkel
Daniel H. Labby
Eugene M. Landis
Milton Landowne
Daniel L. Larson
Willoughby Lathem
Henry D. Lauson
Paul H. Lavietes
H. Sherwood Lawrence
Stanley Melvin Levenson
Marvin F. Levitt
Joseph L. Lilienthal, Jr.
Charles W. Lloyd
Victor Lorber
Frank Lowell
Maclyn McCarty
Currier McEwen
Walsh McDermott
Colin M. MacLeod
Paul Marks
Samuel P. Martin
J. L. Melnick
Albert I. Mendeloff

Milton Mendlowitz
John P. Merrill
Jack Metcoff
Karl Meyer
Max Michael
Max Miller
I. Arthur Mirsky
Wilfred F. H. M. Mommaerts
Carl Moore
Francis D. Moore
Hurley W. Motley
Gilbert H. Mudge
Nicholas Myant
J. D. Myers
J. V. Neel
Norton Nelson
Elliot V. Newman
R. D. Olson
Jack Orloff
John R. Pappenheimer
Emanuel M. Papper
Arthur K. Parpart
Arthur J. Patek
M. Pearlmutter
George A. Perera
Gregory Pincus
Kermit L. Pines
Robert F. Pitts
Myron Prinzmetal
Edith H. Quimby
Charles A. Ragan, Jr.
Elaine P. Ralli
Henry T. Randall
Helen M. Ranney
E. B. Reeve
Dickinson W. Richards, Jr.
Richard L. Riley
Jack D. Rosenbaum
I. N. Rosenberg
Joseph F. Ross
Grace M. Roth
Mitchell I. Rubin
Jonas E. Salk
Victor Sborov
I. Herbert Scheinberg
George Schreiner
William B. Schwartz
David Seegal
Walter H. Seegers
Henry A. Shenkin
Nathan W. Shock
Ephraim Shorr
Clement A. Smith
Emil L. Smith
H. P. Smith
Robert Soberman
Maurice Sokolow
Theodore Spaet
Warren M. Sperry
Malcolm Mc. Stanley
Fredrick J. Stare
J. Murray Steele
DeWitt Stetten
Maurice B. Strauss
Roy C. Swan, Jr.
John V. Taggart
John H. Talbott
Lewis Thomas
William S. Tillett
Daniel C. Tosteson
Joseph C. Turner
Frank H. Tyler
William N. Valentine
H. B. Van Dyke
Donald D. Van Slyke
Eleanor H. Venning
Wade Volwiler
William M. Wallace
Christine Waterhouse
Cecil J. Watson
Ruth Janet Watson
Louis Weinstein
Louis G. Welt
Sidney C. Werner
Laurence G. Wesson, Jr.
John R. West, III
Raymond E. Weston
Abraham G. White
James Laverre Whittenberger
Robert W. Wilkins
C. F. Wilkinson
Hildegarde Wilson
M. M. Wintrobe
W. Barry Wood, Jr.
Theodore E. Woodward
Rosalyn S. Yalow
Lawrence E. Young
Paul Zamecnik
Kenneth L. Zierler
Morris Ziff

THE PREVENTION OF ACTH-INDUCED SODIUM RETENTION BY THE USE OF POTASSIUM SALTS: A QUANTITATIVE STUDY

By GRANT W. LIDDLE,[1] LESLIE L. BENNETT, AND PETER H. FORSHAM

(From the Metabolic Unit for Research in Arthritis and Allied Diseases, and the Departments of Medicine and Physiology, University of California School of Medicine, San Francisco and Berkeley, Calif.)

(Submitted for publication June 4, 1953; accepted August 3, 1953)

Administration of large doses of either corticotropin (ACTH) (1) or the adrenal steroids (2–5) to human subjects ordinarily results in an initial phase of sodium retention which is often accompanied by increased excretion of potassium in the urine and by a decrease in intracellular potassium (6). In order to minimize potassium depletion, it has become common practice to add supplements of potassium salts to the diets of patients receiving large doses of ACTH or cortisone. The use of such supplements was also found to be extremely effective in overcoming the sodium retention induced by ACTH and by cortisone (7). The present study was designed to clarify the mechanism of the sodium diuresis which follows the ingestion of large amounts of potassium salts.

SUBJECTS AND METHODS

Thirty experiments were carried out in 14 subjects. Seven subjects were men, seven woman. Seven subjects had rheumatoid arthritis, one disseminated lupus erythematosus, one dermatomyositis, one gout, one multiple sclerosis, one hyperinsulinism, one Cushing's syndrome, and one no demonstrable disease. No subject with evidence of disease of the liver, kidneys, or of the pituitary or adrenal glands was included in this series.

Throughout the course of the study each subject was maintained on a constant diet. Total urinary output was collected. When appropriate, specimens of feces, saliva, and blood were taken for electrolyte determination. In most experiments, potassium salts were administered orally in small divided doses; in a few cases, the potassium salt was given by continuous intravenous infusion. ACTH was administered intramuscularly, either in aqueous solution every six hours or in 16 per cent gelatin every 24 hours. When either cortisone acetate or hydrocortisone was used, the daily dose was divided into four equal parts administered orally every six hours.

The following laboratory methods were employed: Sodium and potassium determinations were made, using a flame photometer (Perkin-Elmer Model 52-A) with lithium as an internal standard. Chloride was determined by the method of Schales and Asper (8, 9). Inorganic phosphorus was determined by the method of Fiske and Subbarow (10). Urinary ammonia was determined by the method of Folin and Bell (11). Urinary pH was estimated on freshly voided specimens by means of nitrazine paper. Urinary titratable acidity[2] was determined by an adaptation of the method of Henderson and Palmer (12). In preparation for carbon dioxide-plus-bicarbonate determinations, urine was collected under oil in small amounts of carbon dioxide-poor sodium hydroxide solution; carbon dioxide-plus-bicarbonate was then determined on a Van Slyke and Neill manometric blood gas apparatus. Urine specimens for all other chemical determinations were collected in chemically clean glassware containing a known excess of sulfuric acid.

RESULTS

Representative results obtained in each category of experiments are as follows:

The regularity with which administration of ACTH results in retention of sodium in human subjects is illustrated in Figure 1. Subject A. A. received five courses of treatment, each employing a different preparation of ACTH. Retention of sodium occurred during administration of each preparation. Sodium excretion tended to return to control levels after the initial three or four days of treatment, but a frankly negative sodium balance was never observed as long as effective dosage of ACTH was maintained. The abrupt withdrawal of each preparation was followed by a marked diuresis of sodium. In our experience this pattern of sodium retention occurs invariably

[1] U. S. Public Health Service, Post-doctorate Fellow in Arthritis and Metabolic Diseases.

Present address: The Clinical Center, National Heart Institute, Bethesda 14, Md.

[2] The term "titratable acidity" as employed in this report actually refers to "titratable acidity minus carbon dioxide," inasmuch as carbon dioxide was driven off by "boiling" the urine under reduced pressure before titrating to a pH of 7.4.

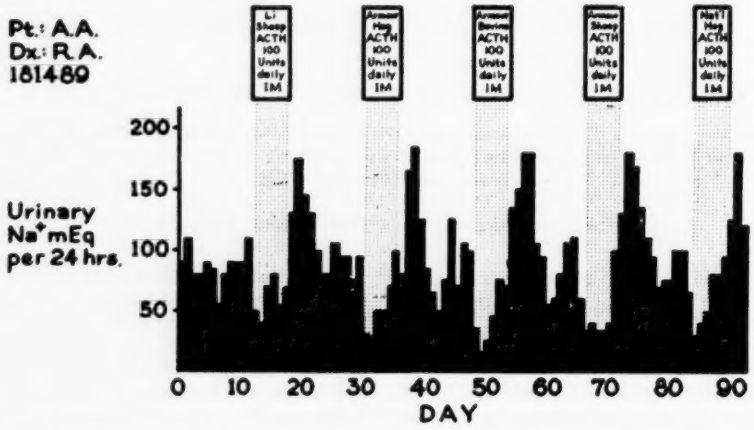


FIG. 1. URINARY SODIUM AS INFLUENCED BY ADMINISTRATION AND WITHDRAWAL OF FIVE DIFFERENT PREPARATIONS OF ACTH IN SUBJECT A. A. WHILE RECEIVING A CONSTANT DIET

in subjects with normal adrenals during continuous treatment with ACTH. The pattern may be profoundly modified, however, when potassium therapy is superimposed upon ACTH therapy.

Typically, the administration of potassium acetate results in sodium diuresis for approximately two days. Sodium excretion then returns to control levels, and there may even be a phase of sodium retention despite continued administration of the potassium salt. The sodium retention produced by ACTH can be completely prevented by simultaneous administration of large doses of potassium acetate (Figure 2). Subject L. G., after a control period of six days, was given potassium acetate, 360 mEq. daily, for a period of 12 days. This particular experiment was atypical in that only a minimal sodium diuresis occurred during the first two days of potassium administration. When ACTH was started on the seventh day of potassium administration, there was no evidence whatever of sodium retention. That potassium acetate had actually prevented the sodium-retaining effect of ACTH became apparent on the nineteenth day of the study, when the potassium salt was withdrawn. At this time the unopposed influence of ACTH became manifest, and the subject entered a phase of marked sodium retention. Finally, on the twenty-fifth day of the study, ACTH was withdrawn and a marked diuresis of sodium ensued.

The complete reversal of ACTH-induced sodium retention by the administration of potassium acetate is illustrated in Figure 3. Subject R. D., after an initial control period, was treated with 25 international units of ACTH, injected intramuscularly every six hours, and the expected phase of sodium retention was readily apparent. On the seventh day of ACTH therapy, the administration of potassium acetate was begun, and despite the fact that ACTH was continued, a striking sodium diuresis occurred, which resulted in a loss of more sodium than had been retained previously. It will be noted that the natriuretic effect of potassium acetate was maximal for only two days and disappeared after four days. After ACTH was withdrawn on the nineteenth day of the study, the sodium loss which usually follows sudden withdrawal of this hormone was hardly preceptible. Finally, on the twenty-fifth day of the study, potassium acetate was withdrawn, and a phase of almost complete sodium retention ensued. A reactive rebound phenomenon is well illustrated in this study: First, the sodium loss which occurred when potassium was administered was greater in magnitude than the preceding ACTH-induced sodium retention; second, the sodium retention which followed withdrawal of potassium exceeded in magnitude the sodium loss which occurred when potassium was administered.

Data presented in Figure 4 show that potassium acetate will also induce sodium diuresis in subjects not receiving ACTH. Subject A. A., who had

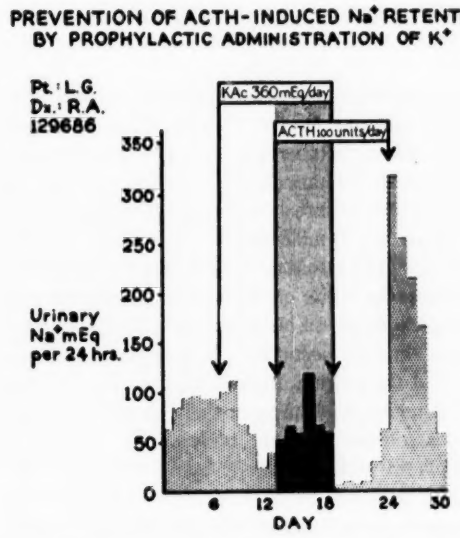


FIG. 2. URINARY SODIUM AS INFLUENCED BY POTASSIUM ACETATE AND ACTH, ADMINISTERED SEPARATELY AND SIMULTANEOUSLY TO SUBJECT L. G. WHILE RECEIVING A CONSTANT DIET

not been treated previously with ACTH, showed a moderate loss of sodium when he received 360 mEq. of potassium acetate in one day. On the following days, sodium was retained. Subsequently, this subject was given the same amount of potassium acetate plus 100 units of ACTH for one day, and a similar sodium diuresis occurred. This indicates that the natriuretic effect of potassium acetate is not limited to subjects receiving corticotherapy.

It will be noted, however, that the sodium diuresis in subject A. A. was much less in magnitude than that observed in other subjects given potassium acetate in comparable doses, most likely because this subject, not having received ACTH, had not retained an excess of sodium before the potassium was administered. As shown in Figure 5, it appears that the degree of sodium loss which occurs when potassium acetate is given is proportional to the body stores of sodium. For 17 days, subject N. C. was given a diet in which sodium intake was severely restricted (6 mEq. per day). During the first six days, urinary excretion of sodium gradually came into equilibrium with sodium intake. Administration of ACTH from the seventh day on resulted in little or no sodium retention. When potassium acetate (360 mEq. per day) was added to the ACTH regimen from the eleventh through the fourteenth days, there was a slight but definite loss of sodium. Withdrawal of potassium was followed by a return to sodium equilibrium. Thus, administration of potassium acetate induces a negative sodium balance, even when not preceded by a phase of sodium retention; the sodium diuresis, however, is less intense when there has not been a preceding phase of sodium retention.

A number of theoretically possible mechanisms were tested in an effort to explain the natriuretic action of potassium salts. Is the natriuretic action a function of the potassium ion alone, or is it influenced by the type of anion administered with the potassium? The fact that potassium salts other than potassium acetate are also effective in producing sodium diuresis is illustrated by the experiments outlined in Tables I, II, and III. Subject V. N. (Table I) had been given intramuscular injections of highly purified ACTH gel, 40 international units daily, for a month by the time this study was begun and had presumably already passed through the early sodium-retaining phase. When

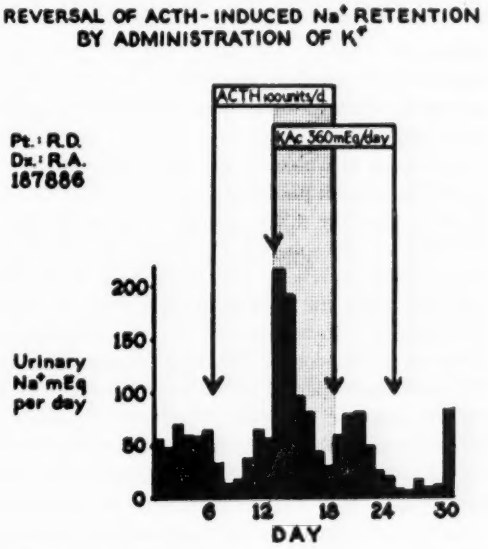


FIG. 3. URINARY SODIUM AS INFLUENCED BY ACTH AND POTASSIUM ACETATE ADMINISTERED SEPARATELY AND SIMULTANEOUSLY TO SUBJECT R. D. WHILE ON A CONSTANT DIET

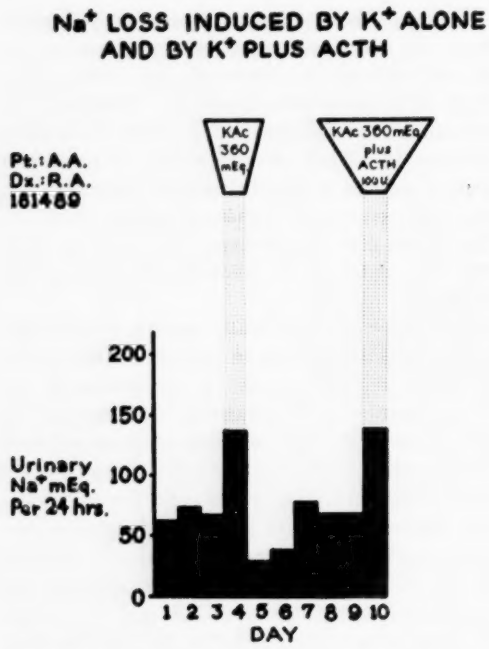


FIG. 4. URINARY SODIUM AS INFLUENCED BY POTASSIUM ACETATE ADMINISTERED ALONE AND IN CONJUNCTION WITH ACTH WITHOUT A PRECEDING PHASE OF ACTH-INDUCED SODIUM RETENTION (SUBJECT A. A.)

potassium chloride, 360 mEq. per day, was administered for six days, a very large loss of sodium occurred. Withdrawal of potassium chloride again permitted sodium retention.

As shown in Table II, the natriuretic effect of neutral potassium phosphate (pH 7.0) may not be obvious when the salt is administered orally, perhaps because phosphate given orally in such large doses causes diarrhea. But natriuresis is readily apparent when the potassium phosphate is given parenterally. In subject N. C., the continuous intravenous infusion of 360 mEq. of potassium in this form for a period of 15 hours resulted in a definite increase in urinary sodium. Similarly, as shown in Table V, the intravenous administration of 180 mEq. of potassium phosphate (pH 7.5) to a patient with hypokalemia due to Cushing's syndrome resulted in a striking sodium diuresis even though the serum potassium never reached normal levels.

Inasmuch as potassium acetate, potassium chloride, and potassium phosphate are all natriuretic agents, it appears that an excess of the potassium ion *per se* may specifically induce sodium excretion.

Is the sodium loss based upon an "osmotic diuresis"? Subject R. Do. (Table III), while receiving no corticotherapy, was given 720 milli-osmols of potassium chloride in one day, and a definite sodium diuresis occurred. One week later he was given 720 milli-osmols of potassium acetate in one day, and a similar sodium diuresis occurred. Subsequently, while on the same regimen, he was given 1440 milli-osmols of urea in one day, and no sodium diuresis occurred. It is apparent, therefore, that the sodium diuretic efficacy of potassium salts is not dependent upon their osmotic activity.

Does sodium leave the body simply because it is displaced within the body by an alternative cation, potassium? Figure 6 presents a comparison of sodium and potassium balances during a typical experiment. During the initial control period, the subject (R. D.) was in approximate sodium and potassium equilibrium. Administration of ACTH resulted in a decrease in urinary excretion of so-

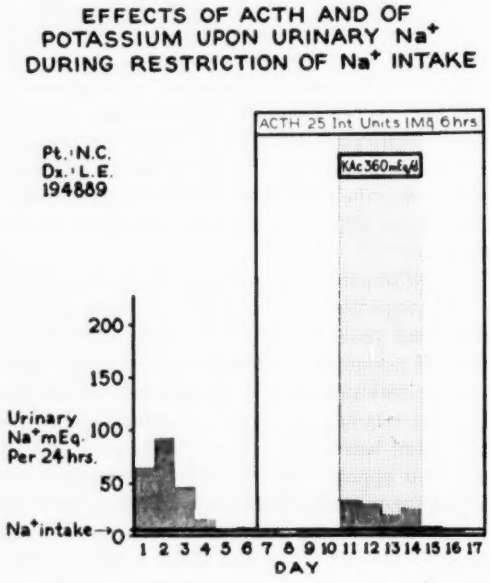

EFFECTS OF ACTH AND OF POTASSIUM UPON URINARY Na+ DURING RESTRICTION OF Na+ INTAKE
ACTH 25 Int. Units IMQ 6 hrs.
KAc 360 mEq/d
Pt.: N.C.
Dx.: L.E.
194889
200
150
Urinary Na+ mEq. Per 24 hrs.
100
50
Na+ intake → 0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
DAY

FIG. 5. URINARY SODIUM AS INFLUENCED BY ACTH AND POTASSIUM ACETATE IN SUBJECT N. C. WHILE ON A DIET CONTAINING 6 MEQ. OF SODIUM IN AN ATTEMPT TO PRECLUDE SODIUM RETENTION DURING ACTH ADMINISTRATION

TABLE I

The effects of potassium chloride on urinary electrolytes

Pt. V. N., No. 199815. White, female, 36 years. Dermatomyositis

			24-Hour urinary						Serum				Salivary	
Day	Treatment		Na (*mEq.*)	K (*mEq.*)	Cl (*mEq.*)	P (*mM*)	Titr. ac. (*mEq.*)	NH_3 (*mM*)	Na *mEq./L.*	K *mEq./L.*	CO_2 *mEq./L.*	Cl *mEq./L.*	Na *mEq./L.*	K *mEq./L.*
1–30	ACTH daily													
31	ACTH-gel 40 Int. Units i.m. daily		74	115	95	30	27	83						
32	ACTH-gel 40 Int. Units i.m. daily		66	110	86	29	22	92						
33	ACTH-gel 40 Int. Units i.m. daily		76	108	90	30	26	82						
34	ACTH-gel 40 Int. Units i.m. daily		75	102	95	31	33	83						
35	ACTH-gel 40 Int. Units i.m. daily		73	123	95	27	24	96						
36	ACTH-gel 40 Int. Units i.m. daily		82	108	107	30	0	80	138	2.7	36	95	7	29
37	ACTH-gel 40 Int. Units i.m. daily	KCl, 27 Gm. orally, daily	228	306	296	18	−128	13						
38	ACTH-gel 40 Int. Units i.m. daily	KCl, 27 Gm. orally, daily	220	436	481	24	−33	25					7	30
39	ACTH-gel 40 Int. Units i.m. daily	KCl, 27 Gm. orally, daily	126	461	407	25	11	30	140	4.4	28	106		
40	ACTH-gel 40 Int. Units i.m. daily	KCl, 27 Gm. orally, daily	66	457	418	27	31	34						
41	ACTH-gel 40 Int. Units i.m. daily	KCl, 27 Gm. orally, daily	152	432	482	25	26	25						
42	ACTH-gel 40 Int. Units i.m. daily	KCl, 27 Gm. orally, daily	120	444	455	25	20	21	142	4.8	27	101	6	29
43	ACTH-gel 40 Int. Units i.m. daily		42	187	181	31	50	32						
44	ACTH-gel 40 Int. Units i.m. daily		21	75	49	24	40	58	139	3.4	31	94	5	28
45	ACTH-gel 40 Int. Units i.m. daily		42	98	105	25	35	81						
46	ACTH-gel 40 Int. Units i.m. daily		72	99	105	24	26	84	143	3.1	30	94		
47	ACTH-gel 40 Int. Units i.m. daily		82	109	100	26	25	96						
48	ACTH-gel 40 Int. Units i.m. daily		74	98	95	28	20	80	140	3.1	35	96	6	32

dium and a positive sodium balance, whereas the over-all potassium balance did not change. When large amounts of potassium acetate were administered, there was an initial phase of potassium retention concurrent with a striking loss of sodium. However, during this initial phase the absolute magnitude of the sodium loss was much greater than the magnitude of the potassium retention. Thus, the sodium loss could only be partially explained in terms of its displacement by potassium. It will be noted also that following the withdrawal of potassium acetate, the sodium retention was far greater in magnitude than the simultaneous potassium loss. This indicates that the administration or withdrawal of potassium influences the excretion of sodium to an extent which exceeds the direct exchange of one cation for the other.

Does potassium cause an increase in sodium excretion by inhibiting the cation exchange mechanism for acidification of urine? Subject L. G. (Table IV), who received ACTH in constant doses throughout the course of this study, was treated for six days with potassium acetate, 360 mEq. per day. During this six-day period the excretion of titratable acid ceased entirely, and the excretion of ammonia was sharply reduced. Presumably, complete inhibition of cation exchange in the renal tubules would result in a disappearance of titratable acid and ammonia from the urine and an equivalent increase in urinary sodium (13). But,

TABLE II

The effect of neutral potassium phosphate on urinary electrolytes

Pt. N. C., No. 194889. White, female, 33 years. Disseminated lupus erythematosus

Day	Treatment		24-Hour urinary					
			Na (*mEq.*)	K (*mEq.*)	Cl (*mEq.*)	P (*mM*)	Titr. ac. (*mEq.*)	NH_4 (*mM*)
1–6	Hydrocortisone 20 mg. orally every 6 hours							
7	Hydrocortisone 20 mg. orally every 6 hours		80	55	82	21	21	47
8	Hydrocortisone 20 mg. orally every 6 hours		79	58	83	22	19	46
9	Hydrocortisone 20 mg. orally every 6 hours		82	51	88	21	23	48
10	Hydrocortisone 20 mg. orally every 6 hours		77	52	81	25	17	45
11	Hydrocortisone 20 mg. orally every 6 hours	KH_2PO_4 12.2 Gm. K_2HP_4 23.5 Gm. (orally)	125	217	131	65	34	29
12	Hydrocortisone 20 mg. orally every 6 hours		89	80	60	45	31	37
13	Hydrocortisone 20 mg. orally every 6 hours		73	65	73	37	25	38
14	Hydrocortisone 20 mg. orally every 6 hours		61	72	54	33	36	53
15	Hydrocortisone 20 mg. orally every 6 hours		58	68	55	27	27	56
16	Hydrocortisone 20 mg. orally every 6 hours		53	62	47	24	18	47
17	Hydrocortisone 20 mg. orally every 6 hours	KH_2PO_4 12.2 Gm. K_2HP_4 23.5 Gm. (intravenously)	176	306	180	236	62	44
18	Hydrocortisone 20 mg. orally every 6 hours		57	109	72	47	38	45
19	Hydrocortisone 20 mg. orally every 6 hours		40	80	62	42	33	47

it will be noted that in this patient the absolute magnitude of the cation exchange performed by the kidneys during pre-potassium periods was not more than 65 mEq. per day (20 mEq. as titratable acidity plus 45 mEq. as ammonia). On the first day of potassium administration, the negative sodium balance was approximately 160 mEq. per day. Therefore, even complete inhibition of the renal tubular cation exchange mechanism for acidification of the urine cannot account for the magnitude of the natriuretic effect of potassium if it is assumed that titratable acid-ammonia excretion is a valid measure of tubular $H^+ - Na^+$ exchange.

Over-all changes in the excretion of electrolytes during administration of large amounts of potassium salts were studied in six subjects. Table I, II, and IV summarize data obtained in representative studies of three subjects. These subjects had been receiving ACTH or hydrocortisone, and, by the time the study was initiated, had already passed through the initial phase of sodium retention and were in approximate sodium equilibrium. Potassium chloride was given to one subject, potassium phosphate to the second, and potassium acetate to the third. All three subjects lost sodium initially when potassium was given, and all retained sodium after potassium was withdrawn. In all studies the pH of the urine increased during administration of potassium; this effect was most marked with potassium acetate and least marked with potassium phosphate. When potassium chloride or potassium acetate was given, urinary ammonia and titratable acidity became negligible, urinary phosphorus decreased sharply, and urinary carbon dioxide-plus-bicarbonate increased markedly. With potassium phosphate, however, urinary titratable acidity-plus-ammonia actually increased.

Interestingly, in most of these experiments loss of sodium was greater than loss of chloride when either potassium acetate or potassium chloride was used. For instance, in subject L. G. administration of potassium acetate resulted in loss of both

TABLE III

Comparison of the effects of urea and potassium salts on urinary electrolytes

Pt. R. Do., No. 130120. White, male, 31 years. Gout

Day	Treatment	24-Hour urinary						
		Na (*mEq.*)	K (*mEq.*)	Cl (*mEq.*)	P (*mM*)	Titr. ac. (*mEq.*)	NH_3 (*mM*)	N (*Gm.*)
1–3	Equilibration period							
4	Control period	99	85		26			
5	Control period	98	80	107	24			
6	Control period	118	73	120	23			
7	Control period	110	82	122	23			
8	Control period	111	81	124	23	17	27	
9	KCl, 27 Gm. orally	176	273	358	20	11	16	
10	Control period	127	196	282	23	25	22	
11	Control period	117	117	151	33	33	26	
12	Control period	114	96	128	30	29	25	
13	Control period	117	97	131	27			
14	Control period	116	86	129	27	24	22	
15	Control period	120	77	125	26	25	22	
16	K Acetate, 36 Gm. orally	199	288	227	16	−108	8	
17	Control period	89	150	74	26	−34	7	
18	Control period	108	111	93	32	3	11	15
19	Control period	110	88	124	26			16
20	Control period	109	101	119	31			15
21	Control period	105	101	125	29			17
22	Control period	98	107	114	29			17
23	Urea, 85 Gm. orally	117	96	138	28			48
24	Control period	96	87	111	27			23
25	Control period	113	83	120	27			20

sodium and chloride, but the magnitude of the sodium loss was much greater than that of the chloride loss. When subject V. N. was given potassium chloride, there was a net retention of both potassium and chloride, associated with a marked loss of sodium. Potassium phosphate, however, administered intravenously to subject N. C., resulted in loss of both sodium and chloride in approximately equal quantities.

Although the administration of potassium chloride tends to correct the hypokaliemic alkalosis of Cushing's syndrome (see Table I), the natriuretic action of potassium is not dependent upon the presence of either alkalosis or hypokaliemia. Of the present series of subjects, all of whom showed natriuretic responses to potassium, only two had hypokaliemic alkalosis (V. N. and C. K.).

The urine is only one of many body fluids whose electrolyte composition is affected by adrenal steroids. The sodium-potassium ratio of saliva, for example, may be greatly decreased during treatment with ACTH or cortisone (14). Data presented in Table I show that in subject V. N., the very low salivary sodium-potassium ratio induced by ACTH was not modified by doses of potassium chloride which considerably altered the urinary electrolyte pattern. It would appear, therefore, that potassium does not overcome the effect of ACTH upon the salivary sodium-potassium ratio at a time when it does modify the effect of ACTH upon the urinary electrolyte pattern.

DISCUSSION

Potassium salts tend to reverse many of the effects of adrenal steroids upon electrolyte metabolism. It is conceivable that part of the sodium loss which follows administration of potassium salts is a result of a readjustment of the intracellular electrolyte pattern, *i.e.*, increase in intracellular potassium and decrease in intracellular sodium. This readjustment would be most apt to occur in subjects in whom there had been a prior depletion of intracellular potassium, which could be induced by any

TABLE IV

Effect of potassium acetate on urinary electrolytes

Pt. L. G., No. 129686. White, female, 33 years. Rheumatoid arthritis

Day	Treatment		24-Hour urinary Na (*mEq.*)	K (*mEq.*)	Cl (*mEq.*)	P (*mM*)	Titr. ac. (*mEq.*)	NH_3 (*mM*)
1	ACTH-gel 40 Int. Units i.m. daily		54	98	64	22	21	45
2	ACTH-gel 40 Int. Units i.m. daily		70	92	86	22	13	40
3	ACTH-gel 40 Int. Units i.m. daily		48	83	60	22	17	47
4	ACTH-gel 40 Int. Units i.m. daily		63	83	70	22	16	48
5	ACTH-gel 40 Int. Units i.m. daily	KAc, 36 Gm. orally, daily	220	353	122	11	−307	10
6	ACTH-gel 40 Int. Units i.m. daily	KAc, 36 Gm. orally, daily	140	402	115	14	−269	10
7	ACTH-gel 40 Int. Units i.m. daily	KAc, 36 Gm. orally, daily	130	492	131	17	−236	11
8	ACTH-gel 40 Int. Units i.m. daily	KAc, 36 Gm. orally, daily	118	405	108	16	−288	12
9	ACTH-gel 40 Int. Units i.m. daily	KAc, 36 Gm. orally, daily	136	446	84	21	−323	13
10	ACTH-gel 40 Int. Units i.m. daily	KAc, 36 Gm. orally, daily	146	415	111	25	−296	8
11	ACTH-gel 40 Int. Units i.m. daily		22	123	36	31	23	22
12	ACTH-gel 40 Int. Units i.m. daily		30	93	51	24	12	25
13	ACTH-gel 40 Int. Units i.m. daily		35	110	62	29	11	34
14	ACTH-gel 40 Int. Units i.m. daily		26	73	37	29	7	35

of a number of factors, including corticotherapy. On the other hand, part of the sodium loss which occurs when potassium acetate or potassium chloride is administered appears to result from a direct action of potassium upon the renal tubules. Berliner, Kennedy, and Orloff (15) have postulated that such an action is in the nature of inhibition of the tubular mechanism by which H^+ ions are excreted in exchange for Na^+ ions. If it is assumed that titratable acid-ammonia excretion is a valid measure of tubular $H^+ - Na^+$ exchange, then two lines of evidence indicate that this mechanism cannot account for the entire sodium loss which follows administration of potassium. First, excretion of titratable acid and ammonia may be regarded as one means of conserving an equivalent amount of sodium. Theoretically, complete suppression of the mechanism for excreting titratable acid and ammonia should result in an equivalent increase in sodium excretion. Several of the balance studies showed, however, that the total amount of titratable acid-plus-ammonia excreted during control periods is much less than the sodium loss observed during administration of potassium. Therefore, even complete suppression of titratable acid-ammonia excretion could not account for all of the actual sodium loss. Second, excretion of titratable acid-plus-ammonia became increased when potassium phosphate was administered. Here, there was no reason to believe that the sodium conservation mechanism under question was suppressed. Nevertheless, the fact that a marked sodium loss did occur suggests that there is more involved in potassium-induced natriuresis than suppression of fixed-base conservation mechanisms. In short, to the extent that potassium *does* inhibit $H^+ - Na^+$ exchange in the renal tubules one may expect a resultant natriuresis; however, potassium *also* induces natriuresis under conditions in which $H^+ - Na^+$ exchange might be occurring at a normal or even increased rate.

On the other hand, it could be assumed that the quantity of H^+ ion appearing in the urine either as titratable acid or with ammonia does not actually represent the total amount of tubular $H^+ - Na^+$ exchange but only a minor fraction thereof. This

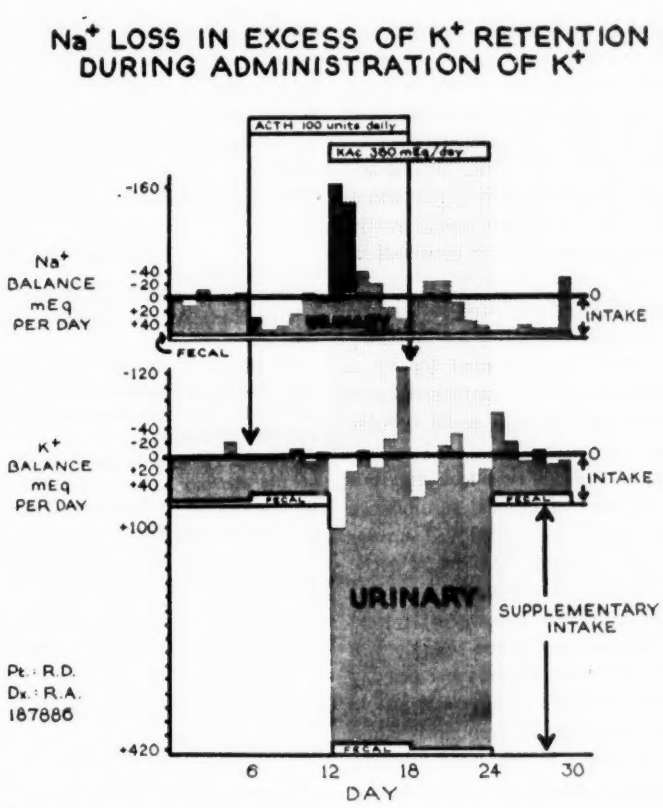


FIG. 6. SODIUM BALANCE AND POTASSIUM BALANCE IN SUBJECT R. D. DURING TREATMENT WITH ACTH AND POTASSIUM ACETATE

Note that the negative sodium balance (black area) is substantially greater than the positive potassium balance (white area) during the first two days of potassium administration.

TABLE V

Effect of potassium phosphate (pH 7.5) on urinary and serum electrolytes

Pt. C. K., No. 81625. White, female, 30 years. Cushing's syndrome

		24-Hour urinary							Serum			
Day	Treatment	Na (*mEq.*)	K (*mEq.*)	Cl (*mEq.*)	P (*mM*)	Titr. ac. (*mEq.*)	NH_3 (*mM*)		Na *mEq./L.*	K *mEq./L.*	Cl *mEq./L.*	CO_2 *mEq./L.*
1	None	64	97	75	32	15	65		140	2.8	92	35
2	None	81	94	103	33	8	60					
3	None	55	93	69	24	12	45		141	2.7	95	36
								Time				
4	KH_2PO_4 2.0 Gm.							0 hr.	143	2.6	94	36
	K_2HPO_4 15.0 Gm.							4 hr.	132	3.3	91	35
	(Intravenously	297	194	240	67	25	41	12 hr.	136	3.6	92	32
	from 0 to 16 hrs.)							24 hr.	134	3.1	94	30
5	None	<1	92	49	27	15	44					
6	None	<1	91	82	21	12	38		138	3.7	92	35

disparity might occur if, as a result of cation exchange, bicarbonate is converted to carbonic acid. The carbonic acid to some extent would become converted to H_2O and CO_2; the CO_2 would tend to diffuse back into the body, thus escaping measurement as titratable acid in the urine. If one accepts this assumption, then the action of potassium in suppressing $H^+ - Na^+$ exchange could easily account for the entire sodium diuresis observed in these studies.

In any case, in all experiments cited in this report the amount of sodium lost under the influence of potassium salts could be accounted for by a summation of two effects: 1) displacement of body Na^+ by K^+, plus 2) suppression of renal tubular $H^+ - Na^+$ exchange.

The complication of sodium retention and potassium depletion becomes of clinical significance only when cortisone or ACTH is employed in relatively large doses (*e.g.*, 100 mg. or more of cortisone daily) and for a relatively long period (*e.g.*, more than one week). When doses greater than this are given and dietary salt is not restricted, a potassium supplement of 200 mEq. or more per day is ordinarily sufficient to prevent sodium retention. Although such doses of potassium frequently cause abdominal cramps or nausea in untreated subjects, even larger amounts are usually well tolerated by subjects receiving corticotherapy. In practice it is best to divide the supplement of potassium into several small doses to be taken throughout the day.

SUMMARY

The oral administration of large doses of either potassium chloride or potassium acetate to human subjects consistently results in a diuresis of sodium. The degree of sodium diuresis is greater in subjects in whom sodium retention has been induced by ACTH or cortisone than in untreated subjects. The administration of potassium chloride and potassium acetate results in a decrease of urinary phosphate, ammonia, and titratable acid and an increase in urinary bicarbonate and chloride. The increase in urinary sodium is proportionately much greater than the increase in urinary chloride. Neutral potassium phosphate, administered intravenously, causes sodium and chloride diuresis, without causing a decrease in urinary titratable acidity-plus-ammonia. It is suggested that two possible mechanisms may account for the natriuretic effect of potassium salts: 1) displacement of body Na^+ by K^+, and 2) suppression of renal tubular $H^+ - Na^+$ exchange. Sodium retention due to therapeutic doses of cortisone or ACTH can be consistently prevented by the daily administration of 200 mEq. or more of potassium.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Robert Berliner, Dr. Isidore Edelman, and Dr. Robert F. Pitts for helpful criticism.

We also gratefully acknowledge the dietetic assistance of Miss Margie Kawasaki and Mrs. Hisaye Mochizuki, as well as the technical assistance of Mr. S. J. Rehfeld and Dr. John Whitney.

The ACTH used in these studies was kindly provided by the Armour Laboratories, the Wilson Laboratories, the National Drug Company, and Dr. C. H. Li. Hydrocortisone was generously supplied by Merck and Company.

REFERENCES

1. Liddle, G. W., Giansiracusa, J. E., Childs, A. W., Island, D., Waechtler, H., and Bennett, L. L., A comparative study of the clinical and metabolic effects of ACTH derived from hogs, sheep, and cattle. J. Lab. & Clin. Med., 1952, **40**, 1.
2. Thorn, G. W., Garbutt, H. R., Hitchcock, F. A., and Hartman, F. A., Effect of cortin upon renal excretion and balance of electrolytes in the human being. Proc. Soc. Exper. Biol. & Med., 1936, **35**, 247.
3. Thorn, G. W., Howard, R. P., and Emerson, K., Jr., Treatment of Addison's disease with desoxy-corticosterone acetate, a synthetic adrenal cortical hormone (preliminary report). J. Clin. Invest., 1939, **18**, 449.
4. Thorn, G. W., Engel, L. L., and Eisenberg, H., The effect of corticosterone and related compounds on the renal excretion of electrolytes. J. Exper. Med., 1938, **68**, 161.
5. Perera, G. A., Pines, K. L., Hamilton, H. B., and Vislocky, K., Clinical and metabolic study of 11-dehydro-17-hydroxy-corticosterone acetate (Kendall Compound E) in hypertension, Addison's disease and diabetes mellitus. Am. J. Med., 1949, **7**, 56.
6. Eliel, L. P., Pearson, O. H., Katz, B., and Kraintz, F. W., Comparison of lymphoid tumor and muscle electrolyte composition in patients treated with ACTH and cortisone acetate. Federation Proc., 1950, **9**, 168.
7. Bennett, L. L., Liddle, G. W., and Bentinck, R. C., Does a large intake of potassium modify the metabolic effects of ACTH in man? J. Clin. Endocrinol., 1953, **13**, 392.

8. Schales, O., and Schales, S. S., A simple and accurate method for the determination of chloride in biological fluids. J. Biol. Chem., 1941, **140**, 879.
9. Asper, S. P., Jr., Schales, O., and Schales, S. S., Importance of controlling pH in the Schales and Schales method of chloride determination. J. Biol. Chem., 1947, **168**, 779.
10. Fiske, C. H., and Subbarow, Y., The colorimetric determination of phosphorus. J. Biol. Chem., 1925, **66**, 375.
11. Folin, O., and Bell, R. D., Applications of a new reagent for the separation of ammonia. I. The colorimetric determination of ammonia in urine. J. Biol. Chem., 1917, **29**, 329.
12. Henderson, L. J., and Palmer, W. W., On the several factors of acid excretion. J. Biol. Chem., 1914, **17**, 305.
13. Pitts, R. F., and Alexander, R. S., The nature of the renal tubular mechanism for acidifying the urine. Am. J. Physiol., 1945, **144**, 239.
14. Frawley, T. F., and Thorn, G. W., The relation of the salivary sodium-potassium ratio to adrenal cortical activity, Proc. Second Clin. ACTH Conf., Blakiston, New York, 1951, vol. 1, p. 115.
15. Berliner, R. W., Kennedy, T. J., Jr., and Orloff, J., Relationship between acidification of the urine and potassium metabolism. Effect of carbonic anhydrase inhibition on potassium excretion. Am. J. Med., 1951, **11**, 274.

SPECIAL NOTICE TO SUBSCRIBERS

Post Offices will no longer forward the Journal when you move.

Please notify The Journal of Clinical Investigation, Business Office, 622 West 168th Street, New York 32, N. Y. at once when you have a change of address, and do not omit the zone number if there is one.

COMPARATIVE STUDIES OF SERUM POLYSACCHARIDES IN RHEUMATOID ARTHRITIS AND DEGENERATIVE JOINT DISEASE

By M. R. SHETLAR, R. W. PAYNE, JANE A. BULLOCK, D. R. PATRICK, ARTHUR A. HELLBAUM, AND WILLIAM K. ISHMAEL

(From the Departments of Biochemistry, Pharmacology, and Medicine, University of Oklahoma School of Medicine, the Research Laboratory of the Veterans Administration Hospital, and the Oklahoma Medical Research Foundation, Oklahoma City, Okla.)

(Submitted for publication June 16, 1953; accepted August 3, 1953)

The origin, physiological role, and metabolism of the serum polysaccharides are largely unknown, and their relationship to changes which occur in connective tissue is not yet firmly established. The occurrence of polysaccharide material in connective tissue has been amply demonstrated by isolation and histochemical methods. Using histochemical methods, an increase of polysaccharide in connective tissue has been noted in many diseases of connective tissue including rheumatic fever, rheumatoid arthritis, and disseminated lupus erythymatosus. Ehrich (1) has recently reviewed the present knowledge of this field. The presence of increased concentrations of polysaccharides in the sera of patients suffering from rheumatic fever (2) and rheumatoid arthritis (3) suggests a possible relationship between serum and tissue polysaccharides in these diseases. Ludwig, Boas, and Soffer (4) demonstrated a possible relationship of connective tissue to serum polysaccharide by showing that an elevation of both the hexosamine of retrobulbular tissue and plasma hexosamine occurred in thyroidectomized guinea pigs after administration of thyrotropic hormone. Catchpole (5) suggests that increased circulating glycoproteins in the blood of tumor animals arise from the ground substance at the site of the invasive growth by a process of depolymerization of tissue glycoprotein. The present study is a more complete analysis of the characteristics of these serum components in rheumatoid arthritis, certain other mesenchymal diseases, and degenerative arthritis, permitting evaluation of the use of serum polysaccharide concentration as a diagnostic aid in differentiating these diseases.

METHODS

The patients described were seen in the arthritis clinic of the University Hospitals. Serum albumin, pseudoglobulin, and euglobulin were fractionated by a modified method of Milne (6) using 21.5 per cent and 26.8 per cent sodium sulfate. Albumin was assumed to be that portion of serum protein which was not precipitated by 26.8 per cent sodium sulfate; euglobulin, that portion which precipitated with 21.6 per cent sodium sulfate; and pseudoglobulin, the difference between the portion precipitated by 26.8 per cent and that precipitated by 21.5 per cent sodium sulfate. Kingsley's method (7) using ethyl ether for the separation of globulin fractions was used for both levels of sodium sulfate. The biuret reagent as described by Weichselbaum and Shapiro (8) was used for the determination of the total serum protein and protein fractions. Mucoprotein was precipitated from sera by the method of Winzler, Devor, Mehl, and Smyth (9). The hexose components of the polysaccharides of each fraction were determined by the tryptophan method of Shetlar, Foster, and Everett (10). As hexosamine does not develop color with tryptophan in this reaction, the method allows an estimate of polysaccharide components other than hexosamine. For simplicity, results obtained by this method will be referred to as "polysaccharide" in this paper. γ-globulin was determined by the method of Jager and Nickerson (11). One hour erythrocyte sedimentation rates (Wintrobe) were conducted by the clinical laboratories of the University Hospitals. The clinical diagnosis was established after several visits to the arthritis clinic where the patient was studied by at least two of the clinical members of the staff. Cases of rheumatoid arthritis were classified as (a) active, if evidence of both joint deformity and moderate or severe joint activity was noted; (b) inactive, if joint deformity, but no activity was present; and (c) early (mild), if no joint deformity, but mild activity was noted.

RESULTS

Serum polysaccharides

Data resulting from the analysis of total serum polysaccharide and mucoprotein are presented graphically in Figure 1. The elevation of serum polysaccharide may be due simply to an increase in serum protein or to an increase of one or more polysaccharide rich protein fractions. To correct for this effect, polysaccharide contents of each frac-

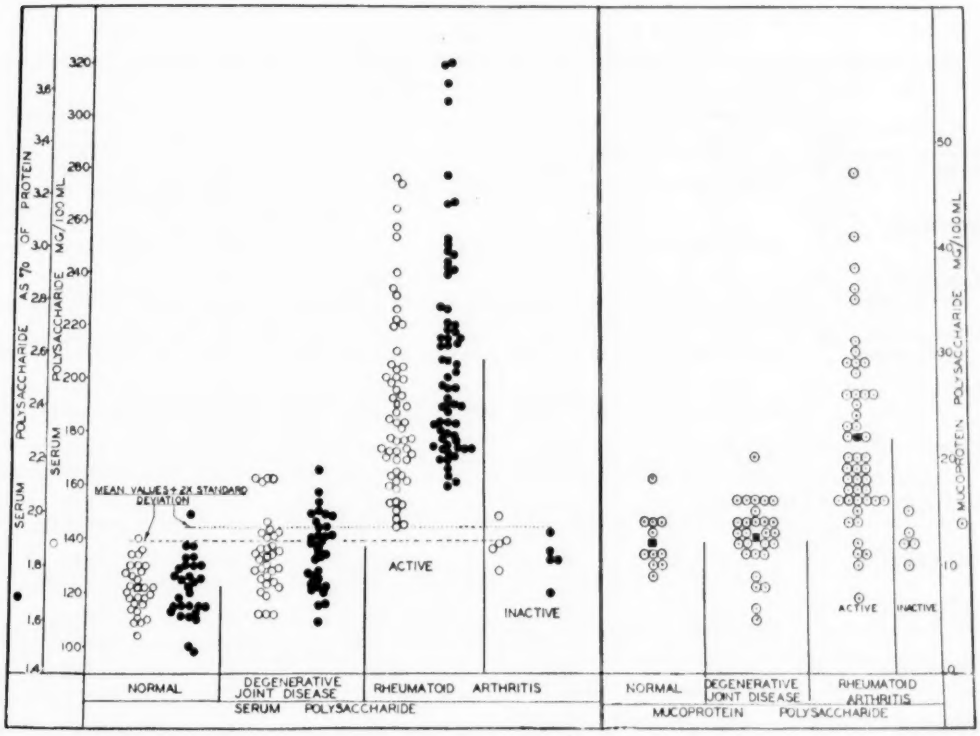


FIG. 1. TOTAL AND MUCOPROTEIN POLYSACCHARIDES OF SERUM IN NORMAL SUBJECTS AND IN PATIENTS WITH DEGENERATIVE JOINT DISEASE AND WITH RHEUMATOID ARTHRITIS

Polysaccharide components exclusive of hexosamine expressed as milligrams per 100 ml. of serum are shown as open circles, while the polysaccharides expressed as a percentage of serum protein are shown as closed circles. Mucoprotein polysaccharide exclusive of the hexosamine is expressed as milligrams per 100 ml. of serum and shown as open circles with a black center. The square in each group represents the average figure for the group.

tion are expressed as percentages of the corresponding protein components and summarized in Table I.

In a preliminary experiment, a known amount of mucoprotein [2] isolated by the method of Winzler, Devor, Mehl, and Smyth (9) was added to an aliquot of previously analyzed human serum. The serum was then fractionated with 26 per cent sodium sulfate as described above. Results were as follows in mg. per 100 ml. of serum:

	Polysaccharide of			
	Total	Albumin	Pseudo-globulin	Mucoprotein
Serum	108	25	17	12
Serum + Mucoprotein	125	42	20	30
Difference	17	17	3	18

[2] Supplied by Dr. Richard J. Winzler, Department of Biological Chemistry, Univ. Illinois College of Medicine.

Apparently in the fractionation procedure with sodium sulfate as described above most of the mucoprotein is included in the albumin fraction. Consequently, results for the albumin polysaccharide were corrected by subtracting the mucoprotein polysaccharide from the original results.

The data were subjected to statistical analysis using the "t" test, where

$$t = \frac{\bar{x}_2 - \bar{x}_1}{S.E.}, \quad \bar{x}_1 \text{ and } \bar{x}_2$$

are means of the two groups being compared, and S.E. is the standard error of the difference of two means. For groups of 28 or more, the standard error was calculated by the formula:

$$S.E. = \left(\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}\right)^{\frac{1}{2}}$$

TABLE I

Percentage of serum polysaccharide in serum protein fractions*

	No. of cases	Polysaccharide of: Total protein Average	S.E.	Albumin Average	S.E.	Pseudoglobulin Average	S.E.	Mucoprotein Average	S.E.	Corrected albumin Average	S.E.
Normal	28	1.71 (1.48–1.95)	.021	0.65 (0.45–0.82)	.017	2.89 (1.89–4.22)	0.11	12 (8–18)	0.8	0.40 (0.28–0.55)	.07
Aged normal	15	1.79 (1.62–2.06)	.025	—		—		—		—	
Degenerative joint disease	28	1.85 (1.59–2.15)	.023	0.77 (0.56–1.10)	.028	2.88 (1.84–4.15)	0.11	13 (6–16)	0.6	0.47 (0.20–0.72)	.03
Rheumatoid arthritis, active	62	2.57 (2.09–3.56)	.051	1.38 (0.72–2.73)	.059	4.14 (1.80–7.74)	0.18	22 (11–47)	1.2	0.74 (0.41–1.47)	.04
Inactive	5	1.82 (1.70–1.92)	.032	0.65 (0.59–0.77)	.036	3.42 (2.73–4.30)	0.21	12 (12–13)	0.1	0.34 (0.30–0.37)	
Early	9	2.03 (1.70–2.27)	.067	0.77 (0.55–0.97)	.016	3.20 (2.70–4.40)	0.10	15 (9–20)	1.3	0.43 (0.28–0.62)	.04
Rheumatoid spondylitis	13	1.90 (1.52–2.29)	.061	0.68 (0.48–0.92)	.041	3.50 (2.57–4.80)	0.16	14 (9–19)	0.1	0.35 (0.26–0.44)	.02
Mixed arthritis†	7	2.15 (1.71–2.57)	.092	0.93 (0.80–1.04)	.046	4.96 (3.33–6.35)	0.48	14 (11–16)	1.2	0.58 (0.55–0.56)	
Gout, active	2	2.65 (2.44, 2.85)		1.30 (1.35, 1.45)		3.13 (2.20, 4.06)		22 (20, 25)		0.86 (0.85, 0.87)	
Gout, inactive	6	2.04 (1.91–2.17)		0.77 (0.60–0.89)		3.80 (3.00–4.76)		15 (11–19)		0.43 (0.25–0.52)	
Psychogenic rheumatism	3	1.72 (1.57–1.99)		0.67 (0.60–0.74)		3.30 (2.47–4.56)		12 (8–18)		0.43 (0.27–0.53)	
Dermatomyositis	4	1.83 (1.77–1.91)		0.68 (0.68–0.69)		3.54 (3.16–3.93)		11 (10–12)		0.42 (0.40–0.52)	
Lupus erythematosus	1	3.40		2.30		5.08		24		1.23	
Periarteritis nodosa	1	1.91		0.91		3.40		13		0.61	
Weber Christian Disease	1	2.13		0.81		3.38		—		—	

* Polysaccharide of each protein fraction expressed as a percentage of the corresponding protein component. Hexosamine component of the polysaccharide complex is not included.

† Both rheumatoid arthritis and degenerative joint disease present.

where σ_1 and σ_2 are standard deviations for the two groups and N_1 and N_2, the number of cases in the respective groups. When less than 28 cases were in either group the following formula was used:

$$\text{S.E.} = \left(\frac{N_1 + N_2}{N_1 + N_2 - 2}\right)^{\frac{1}{2}}\left(\frac{N_1\sigma_1^2 + N_2\sigma_2^2}{N_1N_2}\right)^{\frac{1}{2}}$$

The results of this statistical analysis are summarized in Table II.

An increase of total albumin, pseudoglobulin, and mucoprotein polysaccharide was noted in the sera of patients (aged 8 to 76) with active rheumatoid arthritis. When compared to the patients with degenerative joint disease, highly significant differences between the two groups were noted in all factors. When the degenerative joint disease group (aged 28 to 59) was compared to the normal group (aged 18 to 45) a significant elevation of total and albumin polysaccharide was noted in the group with degenerative joint disease. However, when compared to a group of aged normals (over 60 years) no significant differences were

TABLE II

Summary of statistical comparisons—t values

Comparison	Percentage polysaccharide* of				
	Total	Albumin	Pseudo-globulin	Muco-protein	Corrected albumin†
Rheumatoid A. *vs.* Degenerative joint disease	11.1‡	9.40‡	6.04‡	7.22‡	5.22‡
Normal *vs.* Degenerative joint disease	3.12‡	3.25‡	0.11	0.47	1.37
Aged normal *vs.* Degenerative joint disease	1.47	—	—	—	—
Normal *vs.* Rheum. spondylitis	3.58‡	0.71	5.29‡	1.31	1.18
Normal *vs.* Inactive rheumatoid arthritis	2.04	0.07	4.11‡	—	—

* Polysaccharide of each fraction expressed as a percentage of the corresponding protein component.
† Percentage of polysaccharide in the protein fraction after correction for the mucoprotein.
‡ Significant at the 1 per cent level.

noted. The rheumatoid spondylitis group (aged 20 to 49) was found to have serum polysaccharide levels which were usually within the normal range, although all of this group were in an active phase of the disease. However, considered as a group, significant elevations of total and pseudoglobulin polysaccharide occurred in the rheumatoid spondylitis group. A significant elevation of pseudoglobulin polysaccharide occurred in the patients with inactive rheumatoid arthritis but no other significant differences in this group were noted. Elevations of serum polysaccharides occurred in active gout and disseminated lupus erythematosus, although the number of cases studied was too small to be analyzed statistically. A doubtful elevation was noted in the one case of Weber Christian Disease, while essentially normal values were found in patients with psychogenic rheumatism, dermatomyositis, and periarteritis nodosa (Table I).

Serum proteins

Summary of the fractionated protein values for patients with rheumatoid arthritis, rheumatoid spondylitis, and degenerative joint disease are given in Table III. These results were treated statistically by the "t" test as described above. Changes were observed in serum proteins as determined by salt fractionations in rheumatoid arthritis patients similar to those previously reported using electrophoretic techniques (12). Both pseudo-(α-globulin) and γ-globulin were found to be significantly increased, while albumin was decreased. Smaller but significant elevations of γ-globulin were also noted in the patients with rheumatoid spondylitis and with degenerative joint disease. These results are similar to those reported by Effersoe (13) using a polyfractionation salting-out method with ammonium sulfate.

TABLE III

Summary of the serum protein fractions of arthritis patients as determined by salt fractionation*

	No. of cases	Total	Albumin		Pseudoglobulin	γ-Globulin	A/G ratio	
			Howe	Milne			Howe	Milne
Normal	28	7.08 (6.50–7.73)	5.04 (4.47–5.62)	4.31 (3.43–5.07)	0.73 (.45–1.04)	0.92 (.58–1.55)	2.47	1.55
Rheumatoid arthritis, active	54	7.46† (6.13–8.74)	4.69 (3.44–5.56)	3.78‡ (2.36–4.63)	0.91† (.56–1.59)	1.87† (.81–4.01)	1.71	1.03
Degenerative joint disease	32	7.15 (6.16–8.34)	4.94 (4.22–5.82)	4.09 (3.02–4.82)	0.85 (.51–1.12)	1.34† (.67–2.18)	2.23	1.34
Rheumatoid spondylitis	9	7.57 (6.74–8.82)	5.30 (4.82–6.08)	4.59 (3.89–5.44)	0.71 (.57–.93)	1.47† (.77–2.01)	2.18	1.54

* Expressed as grams of protein per 100 ml. of serum.
† Significantly higher at the 1 per cent level than the corresponding normal value.
‡ Significantly lower at the 1 per cent level than the corresponding normal value.

Sedimentation rates

A summary of erythrocyte sedimentation rates for patients with rheumatoid arthritis, spondylitis, and degenerative joint disease is given in Table IV. Sedimentation rates were generally elevated in the rheumatoid arthritis patients; many of the patients with degenerative joint disease also had elevated sedimentation rates. In order to determine if sedimentation rate and serum polysaccharide concentration reflect similar disease processes in rheumatoid arthritis, the correlation coefficient between these two factors was determined. A correlation coefficient of 0.24 was found between the percentage of polysaccharide in serum protein and the sedimentation rate in patients with rheumatoid arthritis. This coefficient is not statistically significant, that is, it is not significantly different from 0. Thus, no quantitative relationship appears to exist between these factors in this series. This finding recalls the observation of Seibert, Seibert, Atno, and Campbell (14) to the effect that serum polysaccharides do not significantly correlate with sedimentation rates in a series composed largely of patients with tuberculosis.

DISCUSSION

Variation in serum polysaccharides in different mesenchymal diseases is somewhat difficult to evaluate. It seems likely that an elevation of serum polysaccharide may be correlated with either tissue injury, tissue proliferation or both. Shetlar (15) has suggested that an elevation of polysaccharide in the pseudoglobulin and mucoprotein fraction correlates with tissue injury and that an elevation of albumin polysaccharide (after correction for mucoprotein) is related to tissue proliferation. According to these postulates, both inflammation and tissue proliferation occur in many rheumatoid arthritis patients, as 20 out of 43 patients had elevations above the highest normal levels in both albumin and pseudoglobulin polysaccharide components. Only one of 32 patients in the degenerative arthritis group was high enough in total polysaccharide to fall within the range of the rheumatoid arthritis group; however, eight of this group had elevated albumin polysaccharide levels and five had elevated pseudoglobulin polysaccharide levels. The uniformly elevated serum polysaccharide level in rheumatoid arthritis suggests that this assay might be of value as an objective test for evaluating the degree of activity in patients with rheumatoid arthritis. This possibility is currently under investigation.

SUMMARY AND CONCLUSIONS

Comparative polysaccharide studies were made on the sera of patients with rheumatoid arthritis and degenerative joint disease. A limited number of patients with other collagen diseases and with other joint diseases were also studied. The tryptophan method was used to determine the polysaccharide components other than hexosamine of the total serum protein, albumin, pseudoglobulin, and mucoprotein.

Elevations of the polysaccharide of the total protein, albumin, pseudoglobulin, and mucoprotein were found consistently in the sera of patients with rheumatoid arthritis, but not in those with degenerative joint disease. The polysaccharide content of the pseudoglobulin fraction was found to be elevated in inactive rheumatoid arthritis, while other components were unchanged. No significant

TABLE IV

Comparison of serum polysaccharide values with erythrocyte sedimentation rates

	No. of cases	Polysaccharide†	Erythrocyte sedimentation rate* (Wintrobe)		
			Average	Range	Number elevated‡
Degenerative joint disease	28	1.85	26	5–46	20
Rheumatoid arthritis					
Active	62	2.57	40	5–65	58
Early	9	2.03	27	8–41	7
Rheumatoid spondylitis	13	1.90	25	5–38	9

* Expressed in millimeters per hour.
† Percentage of polysaccharide not including hexosamine components found in serum protein.
‡ Number of patients with sedimentation rates above 15 millimeters per hour.

correlation between erythrocyte sedimentation rate and the polysaccharide percentage of serum protein was found in rheumatoid arthritis patients.

Elevations of serum polysaccharides were found in two cases of active gout and in one case of lupus erythematosus, while essentially normal levels were noted in a limited number of patients with inactive gout, dermatomyositis, periarteritis nodosa, and Weber Christian Disease. The use of the determination of serum polysaccharide as a diagnostic aid to distinguish degenerative joint disease from rheumatoid arthritis is suggested.

REFERENCES

1. Ehrich, W. E., Nature of collagen diseases. Am. Heart J., 1952, **43**, 121.
2. Shetlar, M. R., Schmidt, H. L., Jr., Lincoln, R. B., DeVore, J. K., Bullock, J. A., and Hellbaum, A. A., Response of the serum polysaccharide fractions following cortisone treatment of patients with rheumatic fever. J. Lab. & Clin. Med., 1952, **39**, 372.
3. Shetlar, M. R., Shetlar, C. L., Richmond, V., and Everett, M. R., The polysaccharide content of serum fractions in carcinoma, arthritis, and infections. Cancer Research, 1950, **10**, 681.
4. Ludwig, A. W., Boas, N. F., and Soffer, L. J., Role of mucopolysaccharides in pathogenesis of experimental exophthalmos. Proc. Soc. Exper. Biol. & Med., 1950, **73**, 137.
5. Catchpole, H. R., Serum and tissue glycoproteins in mice bearing transplantable tumors. Proc. Soc. Exper. Biol. & Med., 1950, **75**, 221.
6. Milne, J., Serum protein fractionation: A comparison of sodium sulfate precipitation and electrophoresis. J. Biol. Chem., 1947, **169**, 595.
7. Kingsley, G. R., A rapid method for the separation of serum albumin and globulin. J. Biol. Chem., 1940, **133**, 731.
8. Weichselbaum, T. E., and Shapiro, I., A rapid and simple method for the determination of salicylic acid in small amounts of blood plasma. Am. J. Clin. Path. (Tech. Section), 1946, **10**, 42.
9. Winzler, R. J., Devor, A. W., Mehl, J. W., and Smyth, I. M., Studies on the mucoproteins of human plasma. I. Determination and isolation. J. Clin. Invest., 1948, **27**, 609.
10. Shetlar, M. R., Foster, J. V., and Everett, M. R., Determination of serum polysaccharides by the tryptophane reaction. Proc. Soc. Exper. Biol. & Med., 1948, **67**, 125.
11. Jager, B. V., and Nickerson, M., A simple quantitative chemical method for estimating γ-globulin in human serum. J. Biol. Chem., 1948, **173**, 683.
12. Gutman, A. B., The Plasma Proteins in Disease. 15. Rheumatoid Arthritis. Advances in Protein Chemistry, New York, Academic Press, Inc., 1948, **4**, 218.
13. Effersoe, P., Effect of adrenocorticotropic hormone (ACTH) and cortisone on serum proteins in various diseases. Scandinavian J. Clin. & Lab. Invest., 1950, **2**, 265.
14. Seibert, F. B., Seibert, M. V., Atno, A. J., and Campbell, H. W., Variation in protein and polysaccharide content of sera in the chronic diseases, tuberculosis, sarcoidosis, and carcinoma. J. Clin. Invest., 1947, **26**, 90.
15. Shetlar, M. R., The polysaccharides of serum proteins in cancer. Texas Rep. Biol. & Med., 1952, **10**, 228.

ERRATUM

Pulmonary Function in Boeck's Sarcoid, by M. Henry Williams, Jr., J. Clin. Invest., 32: 9, 909, 1953. Table II.

Ventilation
L./min./sq. meter B.S.A.
BTPS
$\dot{V}E$

FRUCTOSE IN THE TREATMENT OF DIABETIC KETOSIS [1, 2]

By J. H. DARRAGH,[3] R. A. WOMERSLEY, AND W. H. MERONEY [4]

(From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.)

(Submitted for publication June 22, 1953; accepted August 11, 1953)

It has long been hoped that a carbohydrate compound could be found which would be utilized effectively in diabetes mellitus without the aid of insulin. Many different hexoses and carbohydrate intermediaries have been tried. However, owing to enzymatic activity in the intestinal mucosa and liver, most of these are converted to glycogen and the net result, in most instances, is comparable to giving similar amounts of glucose (1). Of the hexoses other than glucose, fructose has shown the most promise. The metabolism of fructose differs significantly from that of glucose, despite close similarity in chemical structure. Glucose enters cells by phosphorylation to form glucose-6-phosphate, under the influence of glucokinase. Insulin is necessary to activate this reaction. Fructose enters cells by phosphorylation to form fructose phosphate, under the influence of fructokinase, which does not require activation by insulin (2). One of the blocks in carbohydrate metabolism in diabetes mellitus is the phosphorylation of glucose (3). Other enzyme systems, hexose isomerase and phosphatase, are intact, even in severe diabetes mellitus with ketosis. Fructose should, then, circumvent one of the blocks in carbohydrate metabolism in this disease.

Miller and others (4, 5, 6) have shown that fructose, when administered intravenously, is removed from the blood as rapidly by the diabetic in the absence of insulin as by the normal subject. If fructose could be utilized by patients in diabetic ketosis, at a time when glucose utilization is reduced to a minimum, carbohydrate metabolism would be restored, with a resulting decrease in hepatic ketone body production, decreased ketonemia, and correction of the disturbance in acid-base equilibrium. Fructose might also serve as a convenient vehicle for the administration of water. The purpose of the following study was to determine whether the intravenous administration of fructose has any advantages over the similar use of glucose (7) in the treatment of diabetic ketosis.

EXPERIMENTAL PROCEDURE AND METHODS

Eleven studies have been carried out on five male and five female patients, ranging in age from 16 to 60 years. One patient was treated on two separate occasions. All had the usual clinical and chemical signs of diabetic ketosis. In seven studies there was severe, and in four mild, ketosis.

The studies were divided into two groups. In the first group of four patients, the effects of the intravenous injection of fructose [5] and glucose early in the course of treatment of patients with diabetic ketosis were determined. An initial sample of venous blood was obtained for measurement of electrolytes and blood glucose.

An intravenous infusion of 0.9 per cent NaCl was started and regular insulin was given subcutaneously. Twenty-five Gm. of fructose as a 25 per cent solution were injected intravenously in 10 minutes. Samples of venous blood were obtained every 10 to 15 minutes for two hours after the administration of fructose and analyzed for glucose and fructose. Two of these patients received 25 Gm. of glucose intravenously as a 25 per cent solution in 10 minutes, one before and one after the administration of fructose. For comparison, 25 Gm. of fructose were given in a similar manner to two normal male subjects in the post-absorptive state.

In the second group of seven studies on six patients, larger amounts of fructose were used with insulin and electrolyte solutions in the treatment of diabetic ketosis. The fructose was given as an isotonic solution, 2.5 per cent fructose in 0.45 per cent NaCl. This solution was made by mixing equal quantities of isotonic fructose and saline solutions. Insulin was started at the same time as the infusion in five of the seven studies. In order to determine the effect of fructose administration without insulin, two patients with mild ketosis did not receive insulin until after they had received 50 Gm. of fructose.

Three to nine liters of the fructose-hypotonic saline solution were infused. After the termination of these in-

[1] Presented at the Forty-fifth Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N. J., May 4, 1953.

[2] This work was aided by a research grant from the U. S. Public Health Service.

[3] Work done in part during the tenure of a U. S. Public Health Service Fellowship.

[4] Major, Medical Corps, U. S. Army.

[5] The fructose used in these studies was supplied by E. R. Squibb and Sons.

TABLE I

Analytical data, blood and serum, for patients with diabetic ketosis receiving 2.5 per cent fructose in 0.45 per cent NaCl

Study* no.	Patient Sex	Patient Age	Wt.	Time	Blood Glucose	Blood Fructose	Blood Total ketones	Serum Total hexose‡	Serum Na	Serum K	Serum Cl	Serum CO_2	Serum Tot. prot.
			Kg.	*Hrs.*	*mg./100 cc.*	*mg./100 cc.*	*mM/L.*	*mM/L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*Gm./100 cc.*
1	B38119		50	0.0†	488	0	18	16	134	3.6	102	5.3	8.4
	♀	50		2.7	394	55	14						
				5.5	263	33	5	10	126	3.8	110	12.9	6.2
				12.8§	*184*	*0*	*3*	*6*	*137*	*3.9*	*113*	*16.5*	*6.0*
2	31037		58	0.0	337	0	12	11	126	3.7	103	10.2	7.7
	♀	60		1.3†									
				2.1	300	41	10						
				3.3	260	44	8						
				4.0	244	60	6						
				5.3	192	54	4	8	123	3.7	109	14.6	5.8
				16.0	*214*	*0*	*2*	*7*	*140*	*3.8*	*108*	*15.9*	
3	B28609		44	0.0	443	0	18	15	141	3.4	96	5.0	8.1
	♂	16		0.3†									
				2.3	469	31	16						
				4.9	285	36	12	11	139	3.2	111	8.3	6.8
				14.8	*176*	*0*	*3*	*6*	*140*	*2.9*	*103*	*17.2*	*5.5*
4	A66992		50	0.0†	392	0	16	13	135	4.6	91	13.6	7.3
	♀	47		7.0	208	7	4						
				21.4	*113*	*0*	*2*	*4*	*140*	*4.3*	*98*	*22.3*	*6.1*
5	B55316		50	0.0	286	0	15	9	129	3.6	100	18.4	6.4
	♀	58		1.5†	334	32	13						
				3.5	212	30	10	8	118	2.8	101		5.4
				11.0	*111*	*0*	*5*	*4*	*135*	*3.5*	*98*	*22.0*	*5.6*
6a	B30072		57	0.0	449	0	11	15	130	4.8	93	12.9	7.3
	♀	32		5.0†	508	20	9						
				16.8	*147*	*0*	*2*	*5*	*136*	*4.1*	*104*	*19.4*	*5.2*
6b	B30072		56	0.0†	1780	0	27	59	112	8.4	71	4.4	6.6
	♀	32		3.3	1375	35	22						
				5.1	1045	45	17						
				6.0	854	49	15	30	124	3.4	92	12.2	6.5
				7.8	561	51	11						
				9.6	383	26	8	13	130	3.6	103	19.0	6.2
				11.0	286	27	6						
				18.3	56	12	5	2	131	4.5	106	21.6	6.0

* Study No. 1. Infusion 6.0 liters 2.5% fructose in 0.45% NaCl. 0.2– 6.1 Hrs.
2. Infusion 4.8 liters 2.5% fructose in 0.45% NaCl. 1.3– 5.3 Hrs.
3. Infusion 4.0 liters 2.5% fructose in 0.45% NaCl. 0.3– 4.8 Hrs.
4. Infusion 3.0 liters 2.5% fructose in 0.45% NaCl. 0.5– 7.0 Hrs.
5. Infusion 4.0 liters 2.5% fructose in 0.45% NaCl. 0.0– 3.6 Hrs.
6a. Infusion 5.0 liters 2.5% fructose in 0.45% NaCl. 0.0–15.8 Hrs.
6b. Infusion 9.0 liters 2.5% fructose in 0.45% NaCl. 0.8–18.3 Hrs.

† Insulin therapy started.

‡ The osmotic contribution of the serum total hexose $(mM/L.) = \frac{\text{Blood total hexose (mg./100 cc.)} \times 10}{360 \times 0.85}$.

§ Italics indicate observations after the infusion of fructose hypotonic saline solution.

fusions, the patients received further infusions of saline, glucose, fructose, and oral fluids until they could be maintained on oral intake completely. Potassium chloride was added to the fructose-hypotonic saline solution in five of the seven studies. Four patients received oral as well as parenteral fluids during the infusion. The electrolyte and carbohydrate content of these fluids, as determined from standard food tables (8), are included in the balance data.

Samples of venous blood were obtained before and at intervals during the infusion of the fructose-hypotonic saline solution. A blood sample was obtained at the end of the infusion in all but one study. In the first six studies, a sample of venous blood was obtained several hours after the termination of the fructose-hypotonic saline solution. Urine was collected from the start of treatment. An indwelling catheter was used once; the remainder of the urine specimens were voided. The balance periods outlined in Tables II and III began at the time of the initial

TABLE II

Intake and excretion in urine for patients with diabetic ketosis receiving infusion of 2.5 per cent fructose in 0.45 per cent NaCl

Study no.	Duration	Intake							Excretion in urine							
		Insulin	H_2O	Na	K	Cl	Oral sugar*	I.V. fructose	H_2O	Na	K	NH_3	Cl	Total ketones	Glucose	Fructose
	Hours	*Units*	*Liters*	*mEq.*	*mEq.*	*mEq.*	*Gm.*	*Gm.*	*Liters*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mM*	*Gm.*	*Gm.*
1	0– 5.5	200	5.2	398	47	445	0	129	1.3	62	28		45	58	44.8	5.3
2	0– 5.3	88	5.2	370	80	450	0	120	1.0	16	17	22	24	27	7.3	2.9
3	0– 4.9	150	4.9	393	90	473	22	100	1.2	59	41	39	25	84	55.3	3.6
4	0– 7.0	100	3.2	231	5	231	11	75	1.3	63	45	31	26	71	47.8	4.9
5	0– 3.5	50	4.0	308	0	308	0	100	0.3	18	6		9	29	7.6	2.9
6a	0–13.0	125	5.0	385	40	425	0	125	2.5	98	62	74	53	128	77.1	2.6
6b	0– 6.0	480	3.9	299	0	299	0	97	1.6	21	15	26	14	39	91.7	2.4
	6.0– 9.6	100	2.7	212	52	264	0	69	0.8	13	7	11	9	18	28.5	2.2
	9.6–18.3	0	2.4	183	28	211	0	59	0.6	8	9	18	13	12	13.0	1.9

* Carbohydrate drinks, chiefly sucrose.

blood sample, and terminated at the end of the infusion of the fructose-hypotonic saline solution. Some of the urine collection periods did not coincide with the beginning and end of the balance periods. Appropriate corrections have been made to include only the proper fraction of the total urine.

Serum was analyzed for sodium, potassium, chloride, carbon dioxide content, and total protein; blood for total hexose, fructose, and total ketones; urine for sodium, potassium, ammonia, chloride, total ketones, total hexose, and fructose. Total hexose of whole blood was determined by the method of Nelson (9), using zinc filtrates (10), and in the urine by the method of Somogyi (11). Fructose was determined in zinc filtrates of whole blood, and in urine by the method of Schreiner (12) in the first group of studies, and by the method of Kendrick (13) in the second group. Glucose was computed as the difference between total hexose and fructose. Total ketones in blood and urine were determined by Michaels' modification of the method of Greenberg and Lester (14). Other methods were identical with those previously reported from this laboratory (15, 16, 17).

Balance data were calculated by methods previously reported from this department (18, 19), and by Darrow (20). Cellular transfers of potassium were not corrected for the balance of cellular nitrogen. In calculating the water balance, an allowance of 1 cc. per minute was made for insensible loss. No adjustment was made for the water of oxidation. No corrections have been made for the small amounts of electrolytes lost in the removal of blood

TABLE III

External, extracellular and intracellular balances for patients with diabetic ketosis receiving infusions of 2.5 per cent fructose in 0.45 per cent NaCl

Study no.	Time	Extracellular volume*	External balance					Extracellular balance			Intracellular balance		
			H_2O	Na	K	Cl	Sugar	H_2O	Na	K	H_2O	Na	K
	Hours	*Liters*	*Liters*	*mEq.*	*mEq.*	*mEq.*	*Gm.*	*Liters*	*mEq.*	*mEq.*	*Liters*	*mEq.*	*mEq.*
1	0	7.3											
	5.5	(10.0)	+3.5	+336	+19	+400	+ 79	+2.7	+265	+19	+0.8	+ 71	+ 7
2	0	8.4											
	5.3	(11.6)	+3.9	+354	+63	+426	+110	+3.2	+357	+12	+0.7	− 3	+51
3	0	5.9											
	4.9	(8.7)	+3.4	+334	+49	+448	+ 63	+2.9	+375	+ 8	+0.5	− 41	+41
4	7.0		+1.5	+168	−40	+203	+ 33						
5	0	(9.7)											
	3.5	12.3	+3.5	+290	− 6	+299	+ 90	+2.6	+185	− 1	+0.9	+105	− 5
6a	13.0		+1.7	+287	+22	+372	+ 45						
6b	0	7.4											
	6.0	8.5	+2.0	+278	−15	+285	+ 3	+1.1	+204	−35	+0.9	+ 74	+20
	9.6	9.9	+1.7	+199	+45	+255	+ 38	+1.4	+230	+ 7	+0.3	− 31	+38
	18.3	(11.2)	+1.2	+175	+19	+198	+ 44	+1.3	+180	+ 5	−0.1	− 5	+14

* Calculated from changes in chloride balance, forward or backward, from an assumed extracellular volume (designated by parentheses) of 20 per cent of body weight.

samples, nor in the sweat. Owing to the brevity of the balance periods, and the large transfers involved, the errors introduced by these assumptions are negligible.

RESULTS

A. Intravenous fructose and glucose (Figures 1 and 2)

The effects of the intravenous injection of fructose in four patients with severe diabetic ketosis, and in two normal subjects, are illustrated in Figure 1. The fructose disappeared from the blood at comparable rates in both the patients with diabetic ketosis and the normal subjects. The increase in blood glucose was greater in patients with diabetic ketosis than in normal subjects. In Figure 2 the effects of intravenous fructose and glucose in two patients with diabetic ketosis receiving insulin and intravenous saline are compared. In both patients blood glucose increased considerably after the infusion of glucose, but decreased after the infusion of fructose. The total hexose (glucose plus fructose) of the blood rose much less after the fructose than after the glucose infusion.

B. Patients with diabetic ketosis receiving 2.5 per cent fructose in 0.45 per cent NaCl (Tables I to III)

The initial blood glucose varied from 286 to 1780 mg. per 100 cc. In four studies (Nos. 1, 2, 4, 6b.) there was a progressive fall in blood glucose in spite of the administration of 75 to 225 Gm. of fructose. In one study (No. 3) there was an initial rise in blood glucose when 22 Gm. of carbohydrate [6] were given orally as well as intravenous fructose, and then a gradual fall in blood glucose. Although fructose was infused at rates up to 25 Gm. per hour, the highest concentration of fructose in the blood was 60 mg. per 100 cc.

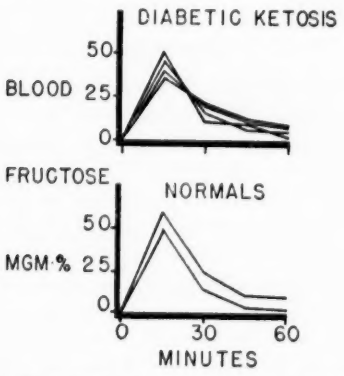


FIG. 1. THE EFFECTS OF INTRAVENOUS INJECTION OF FRUCTOSE IN FOUR PATIENTS WITH SEVERE DIABETIC KETOSIS AND TWO NORMAL SUBJECTS

100 cc. of 25 per cent fructose solution were injected intravenously from 0 to 10 minutes. In the diabetic patients, treatment with regular insulin and intravenous saline had been started prior to 0 minutes.

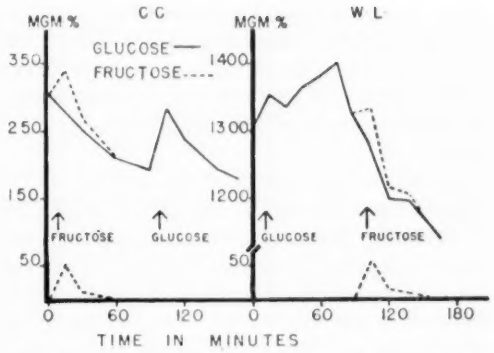


FIG. 2. THE EFFECTS OF INTRAVENOUS INJECTION OF FRUCTOSE AND GLUCOSE IN TWO PATIENTS WITH SEVERE DIABETIC KETOSIS

Blood glucose is illustrated by solid lines; blood fructose by dotted lines. Blood fructose plus blood glucose equals total hexose. 100 cc. of a 25 per cent solution of fructose or glucose were injected intravenously in 10 minutes at the times indicated.

In two studies (Nos. 5, 6a) the patients received 50 Gm. of fructose before receiving insulin. Blood glucose increased during this period, but blood fructose remained low even without insulin. When insulin was added with the fructose, the blood glucose decreased.

Only a small part of the fructose was excreted in the urine (2 to 6 per cent). In most of the studies (Nos. 2 and 5 are the exceptions), much larger quantities of glucose than fructose appeared in the urine. The rate of excretion of glucose varied with the blood glucose and the urine flow, and was independent of the rate of fructose administration.

In every patient there was a steady and rapid fall in the total ketones in the blood. The decrease was greater than that which could be attributed to dilution of the body fluids by the infusion of the fructose-hypotonic saline solution. In two studies

[6] Chiefly sucrose.

(Nos. 5, 6a) the blood ketones decreased both before and after the administration of insulin. The carbon dioxide of the serum was depressed about 1 mEq. per L. for each millimol of ketone bodies, as would be expected from theoretical considerations.

In three studies the initial concentration of sodium in the serum was in the normal range (134 to 144 mEq. per L.) but in the remaining four studies it was below normal. However, these values must be evaluated in terms of the other constituents of the body fluids. The tonicity of the body fluids may be defined as the concentration of those substances which exert an effective osmotic pressure because they are restrained from penetrating cell membranes and therefore can alter the distribution of water between the intracellular and extracellular phases. Under ordinary circumstances extracellular tonicity is almost completely determined by the concentration of sodium salts. Glucose does not enter cells readily, and therefore exerts an effective osmotic pressure. As demonstrated by Seldin and Tarail (21), hyperglycemia contributes significantly to the tonicity of the extracellular fluids. In these studies total hexose of whole blood was measured. The total hexose concentration in serum is about 15 per cent higher than in whole blood due to the distribution of water between cells and serum. In Table I the concentration of total hexose in serum has been expressed in mM per L. $\div$ 2, so that tonicity of the serum can be estimated from 2 $\times$ (total hexose plus sodium). The tonicity of the serum was elevated in four of the studies before treatment (Nos. 1, 3, 4, 6b), and normal in the remaining three (Nos. 2, 5, 6a). Hypertonicity was most marked in the last study (No. 6b) when the initial serum sodium was 112 mEq. per L. and the blood glucose 1780 mg. per 100 cc.

During treatment sodium was infused at a concentration of 77 mEq. per L. In five studies the serum sodium was determined before and after the infusion. In one (No. 6b) the serum sodium increased, in another (No. 3) it did not change, and in three studies it decreased (Nos. 1, 2, 4). In the latter three instances the serum sodium had returned to the normal range by the following morning (Table I—italics). In every instance the serum chloride increased during the infusion of the fructose-hypotonic saline solution.

The initial serum potassium was above normal in only one patient (No. 6b), but fell rapidly with rehydration and acceleration of carbohydrate metabolism. Potassium chloride (7, 22, 24) was administered in five of the studies to prevent the development of hypokalemia (Table II). The urine was always hypotonic with respect to electrolytes (Table II). In every instance there was a significant loss of potassium in the urine, generally less than that of sodium and ammonia. Excretion of chloride was low initially, when there was a marked ketonuria, but increased rapidly with the decrease in the rate of excretion of ketones.

In every instance, a large positive balance of water, sodium, and chloride was achieved during the infusion of the fructose-hypotonic saline solution. The decrease in the concentration of proteins in the serum during the studies reflects correction of the dehydration. In six of the seven studies, sodium chloride was retained as a hypotonic solution (No. 6a is the exception). In the five studies in which the cellular balances have been calculated (Table III), the retained water was distributed chiefly in the extracellular compartment. In each instance there was a significant increase in intracellular water. When potassium chloride was added to the fructose-hypotonic saline solution (Table II), a positive balance of potassium was achieved. With one exception (No. 1), the potassium was distributed chiefly in the intracellular compartment.

DISCUSSION

The derangements of body water and electrolytes in severe diabetic ketosis have been reviewed by several authors (7, 21, 23–25). As a result of hyperglycemia and glycosuria, hyperventilation, and the vomiting which frequently occurs in diabetic ketoacidosis, there is usually a loss of large quantities of intracellular and extracellular water and electrolytes. In most instances these substances are lost in proportions less than their concentration in the body fluids, so that the net result is hypertonicity of the body fluids and cellular dehydration. Seldin and Tarail (21) have demonstrated that the hypertonicity is not apparent if only the concentration of sodium chloride in the serum is considered. Glucose does not enter cells readily and thus contributes to the effective osmotic pressure of the extracellular fluid, which must be

estimated from the concentration of sodium and its salts plus the osmotic contribution of glucose. In the treatment of diabetic ketosis, deficits of water and electrolytes must be replaced. Water is needed in excess of that in isotonic solutions to correct hypertonicity and cellular dehydration, as well as the provision for urine and insensible loss which is increased with the hyperpnea of diabetic ketosis. Infusion of hypertonic electrolyte or glucose solutions will increase the hypertonicity and cellular dehydration. Danowski and his associates (24) found that the infusion of large volumes of isotonic saline can prolong the hypertonicity. For these reasons Butler (25), Seldin and Tarail (21), and Peters (7), have recommended hypotonic electrolyte solutions in diabetic acidosis. There are objections to the rapid infusion of large volumes of hypotonic electrolyte solutions. Hence, glucose has been used as a vehicle for the water so that an isotonic solution could be administered, either by giving isotonic glucose (5 per cent) and isotonic saline separately, or mixed together in equal quantities, 2.5 per cent glucose in 0.45 per cent saline. However, in severe diabetic ketosis, when carbohydrate metabolism is reduced to a minimum, glucose is not an ideal vehicle for water. Up to the present, it has been recommended because none better was available. Glucose administered early in the course of treatment of diabetic ketosis either increases the blood glucose or delays its return to normal (21, 26). The data presented here show that fructose disappears from the blood, even in severe diabetic ketosis, and that the blood total hexose decreases if adequate amounts of insulin are given. Hence, fructose is a better vehicle than glucose for the administration of the excess water required in the correction of the dehydration associated with diabetic ketosis. Other investigators have arrived at the same conclusion (27, 28).

The need for excess water in the treatment of diabetic ketosis is illustrated best in the last study (No. 6b, Tables I–III). This patient received in 18.3 hours 9 liters of water, containing 693 mEq. of sodium and 225 Gm. of fructose. Of the 9 liters, 3.8 liters were retained in the extracellular compartment together with 614 mEq. of sodium (162 mEq. per liter). The remaining 6.2 liters were utilized as hypotonic solutions: intracellular water, 1.1 liters with 38 mEq. of sodium; urine, 3 liters with 42 mEq. of sodium; and 1.1 liters for insensible loss (total electrolyte content less than 10 mEq. per liter).

Fructose circumvents one of the blocks in carbohydrate metabolism in diabetes mellitus (2, 3). It was hoped that fructose would prove to be more antiketogenic than glucose. In these patients treated with insulin and fructose, there was a rapid and steady decrease in blood ketones. When fructose was given without insulin to two patients with mild ketosis (Nos. 5, 6a, Table I), there was a slight decrease in blood ketones, but fructose did not appear to be more antiketogenic than glucose. Rosecan and Daughaday (29) have compared the treatment of diabetic ketosis with insulin and saline, and insulin, saline, and glucose or fructose. They found a more rapid disappearance of the ketosis with insulin plus carbohydrate, but found that fructose was no more antiketogenic than glucose.

Fructose administered intravenously in diabetic ketosis disappears rapidly from the blood. Only small amounts of fructose are excreted in the urine. Miller and his associates (4, 30) have demonstrated that: 1) There is a large hepatic uptake of fructose in diabetic patients deprived of insulin; 2) some of the fructose is converted to glucose by the liver; 3) in severe diabetic ketosis as much as 22 per cent of intravenously administered fructose can be utilized even in the absence of insulin (31); 4) in severe diabetes without insulin, C^{14}-labelled fructose is oxidized to $C^{14}O_2$ at the same rate as in normal subjects (31). The elevation of blood glucose which follows the intravenous administration of fructose is variable. It is greater in patients with diabetes than in normal subjects, and in diabetics it is less with insulin than without insulin. Even in mild diabetic ketosis (Nos. 5, 6a, Table I) fructose without insulin produced an increase in blood glucose.

In the choice of a carbohydrate compound for use in the early treatment of diabetic acidosis, fructose has advantages over glucose. Part of the fructose is utilized when glucose would be excreted quantitatively in the urine. Fructose administered intravenously is removed rapidly from the blood, releasing the water.

There is not an elevation of the blood glucose when adequate amounts of insulin are given. We have started the parenteral fluid therapy of diabetic ketosis with fructose as a 2.5 per cent solu-

tion in 0.45 per cent NaCl so as to administer an isotonic solution early in the course of treatment when there is usually hypertonicity of the body fluids, with some salt, but chiefly water depletion. In a patient with prolonged and severe diabetic acidosis with shock, the blood volume should also be expanded with blood, plasma, or colloid solutions. Once the blood sugar has begun to fall, owing to the acceleration of carbohydrate metabolism, and hypertonicity has been corrected, more concentrated solutions should be used, such as 5 per cent or 10 per cent fructose or glucose, in order to prevent the development of hypoglycemia (*7*, *21*).

CONCLUSIONS

1. Fructose disappears rapidly from the blood in patients with diabetic ketosis, with or without insulin, and at the same rate as in normal subjects.

2. By using fructose instead of glucose, together with adequate amounts of insulin, carbohydrate can be administered early in the course of treatment of diabetic ketosis without increasing the effective osmotic pressure of the extracellular fluid.

3. The dehydration associated with diabetic ketosis can be corrected rapidly by infusing a hypotonic electrolyte solution made up to isotonicity with fructose. Intracellular water is increased early in the course of treatment.

ACKNOWLEDGMENTS

The authors would like to thank Dr. J. P. Peters and Dr. M. Miller for their help in the preparation of this manuscript.

REFERENCES

1. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, ed. 2, Baltimore, The Williams & Wilkins Co., 1946, vol. 1, p. 140.
2. Cori, G. T., Ochoa, S., Slein, M. W., and Cori, C. F., The metabolism of fructose in liver. Isolation of fructose-1-phosphate and inorganic pyrophosphate. Biochim. et Biophys. Acta, 1951, **7**, 304.
3. Chernick, S. S., and Chaikoff, I. L., Two blocks in carbohydrate utilization in the liver of the diabetic rat. J. Biol. Chem., 1951, **188**, 389.
4. Miller, M., Drucker, W. R., Owens, J. E., Craig, J. W., and Woodward, H., Jr., Metabolism of intravenous fructose and glucose in normal and diabetic subjects. J. Clin. Invest., 1952, **31**, 115.
5. Smith, L. H., Jr., Ettinger, R. H., and Seligson, D., A comparison of the metabolism of fructose and glucose in hepatic disease and diabetes mellitus. J. Clin. Invest., 1953, **32**, 273.
6. Weichselbaum, T. E., and Daughaday, W. H., The rate of assimilation of fructose following the intravenous administration to patients with diabetic acidosis and total pancreatectomy. J. Lab. & Clin. Med., 1951, **38**, 958.
7. Peters, J. P., Diabetic acidosis. Metabolism, 1952, **1**, 223.
8. McCance, R. A., and Widdowson, E. M., The Chemical Composition of Foods, Brooklyn, Chemical Publishing Co., 1947.
9. Nelson, N., A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 1944, **153**, 375.
10. Somogyi, M., A method for the preparation of blood filtrates for the determination of sugar. J. Biol. Chem., 1930, **86**, 655.
11. Somogyi, M., Notes on sugar determination. J. Biol. Chem., 1952, **195**, 19.
12. Schreiner, G. E., Determination of inulin by means of resorcinol. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 117.
13. Kendrick, A. B., Determination of fructose in presence of glucose in blood and urine by use of diphenylamine. Federation Proc., 1952, **11**, 239.
14. Michaels, G. D., Margen, S., Liebert, G., and Kinsell, L. W., Studies in fat metabolism. I. The colorimetric determination of ketone bodies in biological fluids. J. Clin. Invest., 1951, **30**, 1483.
15. Hald, P. M., Determinations with Flame Photometer, Methods in Medical Research, The Yearbook Publishers, Inc., 1951, vol. 4, p. 79.
16. Elkinton, J. R., and Taffel, M., Prolonged water deprivation in the dog. J. Clin. Invest., 1942, **21**, 787.
17. Conway, E. J., Microdiffusion Analysis and Volumetric Error, London, C. Lockwood, 1947, p. 357.
18. Elkinton, J. R., and Winkler, A. W., Transfers of intracellular potassium in experimental dehydration. J. Clin. Invest., 1944, **23**, 93.
19. Elkinton, J. R., Winkler, A. W., and Danowski, T. S., Transfers of cell sodium and potassium in experimental and clinical conditions. J. Clin. Invest., 1948, **27**, 74.
20. Darrow, D. C., The retention of electrolyte during the recovery from severe dehydration due to diarrhea. J. Pediat., 1946, **28**, 515.
21. Seldin, D. W., and Tarail, R., The metabolism of glucose and electrolytes in diabetic acidosis. J. Clin. Invest., 1950, **29**, 552.
22. Dyer, W. W., Potassium: Metabolic Aspects (Diabetes) *in* Advances in Medicine and Surgery, Philadelphia, W. B. Saunders Co., 1952, p. 94.
23. Atchley, D. W., Loeb, R. F., Richards, D. W., Jr., Benedict, E. M., and Driscoll, M. E., On diabetic acidosis. A detailed study of electrolyte balances following the withdrawal and reestablishment of insulin therapy. J. Clin. Invest., 1933, **12**, 297.

24. Danowski, T. S., Peters, J. H., Rathbun, J. C., Quashnock, J. M., and Grenman, L., Studies in diabetic acidosis and coma, with particular emphasis on the retention of administered potassium. J. Clin. Invest., 1949, **28**, 1.
25. Butler, A. M., Diabetic coma. New England J. Med., 1950, **243**, 648.
26. Franks, M., Berris, R. F., Kaplan, N. O., and Myers, G. B., Metabolic studies in diabetic acidosis. I. The effect of the early administration of dextrose. Arch. Int. Med., 1947, **80**, 739.
27. Dolger, H., Kupfer, S., Bookman, J. J., and Carr, J., Intravenous fructose in diabetes mellitus: advantages over glucose in the treatment of diabetic emergencies. J. Clin. Invest., 1953, **32**, 562.
28. Miller, M., Murphy, J. R., Craig, J. W., and Woodward, H., Jr., Studies in experimental diabetic acidosis: comparison of the effect of fructose and glucose in the initial hours of treatment. Presented at the 13th Annual Meeting, Am. Diabetes A., New York, N. Y., May 30, 1953.
29. Rosecan, M., and Daughaday, W. H., A comparison of insulin treatment with and without added carbohydrate in human diabetic ketosis. J. Clin. Invest., 1953, **32**, 599.
30. Craig, J. W., Drucker, W. R., Miller, M., Owens, J. E., Woodward, H., Jr., Brofman, B., and Pritchard, W. H., Metabolism of fructose by the liver of diabetic and non-diabetic subjects. Proc. Soc. Exper. Biol. & Med., 1951, **78**, 698.
31. Miller, M., Craig, J. W., Woodward, H., Jr., Owens, J. E., Drucker, W., and Murphy, J., The metabolism of fructose in diabetic subjects. Presented at the 12th Annual Meeting, Am. Diabetes A., Chicago, Ill., June 7–8, 1952.

A QUANTITATIVE STUDY OF THE DIGITOXIN CONTENT OF EDEMA FLUIDS[1]

By SHIRLEY ST. GEORGE, CHARLES F. NAEGELE, FRANK S. FRENCH, RAY H. ROSENMAN, AND MEYER FRIEDMAN

(From the Harold Brunn Institute, Mount Zion Hospital, and the Medical Service, The United States Public Health Service Hospital, San Francisco, Calif.)

(Submitted for publication June 22, 1953; accepted August 11, 1953)

For many years it has been suggested that considerable quantities of digitalis bodies accumulate in edema fluid, that rapid diuresis with mercurial diuretic drugs "liberates" these glycosides into the blood stream as the edema fluids are mobilized and excreted by the kidneys, and that the ensuing "re-digitalization" may lead to manifestations of digitalis toxicity in the already adequately digitalized patient (1). A survey of the literature reveals that this belief is based largely upon clinical impressions rather than upon careful studies of the content of digitalis glycosides actually present in accumulations of edema fluid. The clinical importance of this problem led to the present investigation of the digitoxin content of edema fluids, utilizing microassay techniques now available.

METHODS

Peritoneal, pleural, and subcutaneous edema fluids were removed by paracentesis or through Southey tubes from seven patients with accumulations of edema fluid of hepatic or cardiac origin, and from one patient with pleural effusion secondary to a lymphoma. In six patients fluid was collected prior to the oral administration of 1.2 mg. of digitoxin and again collected from the same site four to seven days later, during maintenance with 0.2 mg. daily dosage. In the remaining two patients, the collection was made only once, following oral digitalization and during maintenance with a daily dose of 0.1 mg. digitoxin. All samples of fluid were collected with toluol as preservative, measured, and then extracted immediately or after short periods of storage at $-20°$ C.

Duplicate aliquots of 10 ml. each were buffered at pH 8 with 0.1 M phosphate buffer and extracted with 25 ml. of methylene chloride by shaking for one hour at room temperature. The flasks were chilled overnight at 4° C., transferred to 200 ml. centrifuge tubes and centrifuged for 30 minutes at 2000 rpm. The aqueous layer was aspirated and the protein film then separated from the methylene chloride layer by filtration. The filtrate was evaporated to dryness; a small amount of ethanol was added at the close of the process to drive off all traces of dichloromethane. The residue was shaken with 2 ml. Tyrode's solution (five times the original concentration) and diluted further if necessary. Bioassay then was performed by the standard embryo duck heart procedure (2, 3). The presence of digitoxin was indicated by a more forceful heartbeat followed by the onset of auriculo-ventricular dissociation (2), changes which are specific for digitalis glycosides in contrast with other effects which are induced by various drugs and electrolytes. Standardization was accomplished by the recovery of 1.0, 0.05, and 0.025 micrograms of digitoxin added to the edema fluids of two non-digitalized patients, and from Tyrode's solution containing 5 mg. of albumin. Re-extraction of either the aqueous or protein layers did not yield further quantities of digitoxin (or digitoxigenin). In all of the cases where fluid samples without digitalis from any source were available, the extract of the fluid caused no irregularities in the embryo duck heart preparation and served as the biological control of the post-digitalization specimens. The sensitivity of the method permitted the identification of amounts of digitoxin as little as 0.0025 micrograms per cubic centimeter of edema fluid (0.25 μg. per cent).

RESULTS

As shown in Table I, no digitoxin was found in the edema fluid collected from the subcutaneous tissues of the ankle in the one patient in which such fluid was obtained. Edema fluids collected from the peritoneal cavities of three other patients failed to show detectable amounts of digitoxin; in three others the ascitic fluid was found to contain traces of digitoxin, varying in concentration from 0.002 to 0.02 micrograms per cubic centimeter of fluid. The single specimen of pleural fluid revealed a digitoxin concentration of 0.005 micrograms per ml.

DISCUSSION

A variable amount of edema fluid is mobilized and excreted by the kidneys when diuresis is induced. For many years it has been assumed that edema fluids of digitalized patients may contain digitalis glycosides (1). This concept has led

[1] Aided by a grant from Eli Lilly and Company.

TABLE I

The digitoxin content of edema fluids of digitalized patients

Patient	Age	Diagnosis	Type of fluid	Volume of fluid (*ml.*)	Period of digitalization (*days*)	Digitoxin content *μg./ml.*
H. C.	47	Hepatic cirrhosis	Ascitic	500	5	0.020
S. S.	64	Hepatic cirrhosis	Ascitic	4700	4	0.003
C. B.	54	Hepatic cirrhosis	Ascitic	20	6	none
A. L.	64	Hepatic cirrhosis and hepatoma	Ascitic	1280	4	none
J. W.	60	Lymphoma	Pleural	500	4	0.005
J. P.	72	Hepatic cirrhosis, atherosclerotic heart disease	Ascitic	2000	6	0.002
G. R.	28	Rheumatic heart disease	Subcutaneous (ankle)	300	Chronic*	none
J. H.	54	Arteriosclerotic heart disease	Ascitic	2400	Chronic*	none

* Received maintenance dose of 0.1 mg. daily. All others received 1.2 mg., followed by daily dose of 0.2 mg.

to the hypothesis that rapid diuresis following the injection of mercurial preparations may introduce excess glycoside into the blood stream and that this "redigitalization" of the already digitalized patient may induce manifestations of digitalis toxicity.

Widespread acceptance of this concept seems based upon two studies. In 1931, Miller and Smith (1) employed the Hatcher cat assay method to study edema fluids obtained from 15 patients and found "digitalis action" in each instance. In 1937, Schnitker and Levine (4) utilized the frog heart to study this problem, finding a "digitalis effect" in 13 of 18 specimens of edema fluid.

The clinical importance of this problem led us to reinvestigate the content of digitoxin in edema fluids, utilizing a microassay technique capable of identifying minute amounts of glycoside. Edema fluids were obtained from peritoneal, pleural, and subcutaneous sites of eight digitalized patients. In six instances, collections were made both before and after digitalization was accomplished. The presence of digitoxin was detected in only four of the eight specimens. In each instance the concentration was less than 0.02 micrograms per ml. of edema fluid. Thus, the maximum content found was of the order of 20 micrograms per liter of fluid. This suggests that the mobilization of even large quantities of edema fluid could not lead to the hypothetical "redigitalization" following rapid diuresis.

Nevertheless, such symptoms as headache, giddiness, weakness, nausea, and even emesis may follow marked diuresis, and may be accompanied by cardiac arrhythmias. It seems likely that these manifestations may be due to the marked alteration of water and electrolyte balance induced by rapid diuresis. In this regard there is increasing evidence of the importance of the potassium ion to manifestations of digitalis toxicity (5, 6). Of particular interest is the recent report of Lown and associates (7) of their finding that mercurial-induced diuresis of the digitalized patient can lead to sufficient loss of potassium ion to change the threshold to the toxic action of digitalis, with ensuing manifestations of digitalis toxicity.

SUMMARY

The content of digitoxin in peritoneal, pleural, and subcutaneous edema fluids was determined by microassay techniques utilizing the embryo duck heart procedure. No digitoxin was detected in specimens removed from four of eight digitalized patients, and only minute amounts were detected in the remaining four specimens. The maximal content of digitoxin was found to be 20 micrograms per liter of fluid.

It was suggested that rapid diuresis of even large quantities of edema fluid cannot lead to "redigitalization" of the already digitalized patient.

REFERENCES

1. Miller, G. H., and Smith, F. M., The presence of digitalis in edema fluid and its possible clinical significance. J. Clin. Invest., 1931, **10**, 666.

2. Friedman, M., and Bine, R., Jr., Employment of the embryonic duck heart for the detection of minute amounts of a digitalis glycoside (Lanatoside C). Proc. Soc. Exper. Biol. & Med., 1947, **64**, 162.
3. Friedman, M., Byers, S. O., and Bine, R., Jr., Rate of disappearance of digitoxin from the blood of man after its parenteral administration. Federation Proc. 1951, **10**, 46.
4. Schnitker, M. A., and Levine, S. A., Presence of digitalis in body fluids of digitalized patients. Arch. Int. Med., 1937, **60**, 240.
5. Sampson, J. J., and Anderson, E. M., The treatment of certain cardiac arrhythmias with potassium salts. J. A. M. A., 1932, **99**, 2257.
6. Lown, B., Salzberg, H., Enselberg, C. D., and Weston, R. E., Interrelation between potassium metabolism and digitalis toxicity in heart failure. Proc. Soc. Exper. Biol. & Med., 1951, **76**, 797.
7. Lown, B., Wyatt, N. F., Crocker, A. T., Goodale, W. T., and Levine, S. A., Interrelationship of digitalis and potassium in auricular tachycardia with block. Am. Heart J., 1953, **45**, 589.

IRON ABSORPTION IN DOGS DURING ANEMIA DUE TO ACETYLPHENYLHYDRAZINE

By W. B. STEWART, PHILIP S. VASSAR, AND ROBERT S. STONE

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, N. Y.)

(Submitted for publication June 25, 1953; accepted August 3, 1953)

The mechanisms controlling the absorption of iron from the gastrointestinal tract have been under investigation for a number of years. It now appears that several factors can increase the absorption of iron above the small amounts normally absorbed. Early observations by Hahn, Bale, Lawrence, and Whipple (1) using radioactive isotopes of iron clearly demonstrated that the normal dog absorbed only small amounts of iron from the gastrointestinal tract, whereas the anemic iron deficient dog was capable of absorbing relatively large quantities of iron. This observation was extended to rats (2) and human subjects (3). It appeared, however, that in spite of adequate iron stores, excessive iron could be absorbed under certain circumstances. Patients with pernicious anemia, hemolytic anemia, and other anemias seemed to absorb more iron than normal when iron balance studies were done with the aid of radioactive isotopes (3). These types of anemia are generally associated with increased rather than decreased stores of iron. Furthermore, in several instances of prolonged anemia of various types which required multiple transfusion of blood over a number of years, there was deposited in the liver considerably more iron than had been administered by transfusion, suggesting an increase in the absorption of iron (4, 5). Studies in pigs deficient in pyridoxine (6) also suggested that the absorption of iron was increased, perhaps in this instance also because of the anemia which is regularly present.

Some of the above mentioned observations suggested to Granick (7) that anemia alone may have been responsible for the increased absorption of iron that was noted. Several objections to this idea are possible, however, since in all instances factors other than anemia have been present. These include abnormal gastric mucosa, deficiencies of iron and vitamins. It was, therefore, considered desirable to approach this problem by studying the influence of a chronic anemia on the absorption of iron in dogs in which these variables were controlled.

The anemia induced by acetylphenylhydrazine seemed an ideal method of conducting such a study. The only known action of this substance is to cause an increased destruction of erythrocytes. The anemia which it causes is of hemolytic type, little loss of essential building blocks for hemoglobin synthesis occurs, and the duration and severity of anemia can be readily controlled.

The experiments reported here indicate that under conditions of prolonged hemolytic anemia the dog absorbs excessive amounts of iron.

METHODS

All dogs were healthy adult mongrels, vaccinated against distemper, and freed of intestinal parasites by a commercial vermifuge. The diet consisted of dog meal supplemented by occasional cans of commercial canned dog meat.

The radioactive iron, a mixture of Fe^{55} and Fe^{59} as $FeCl_3$ was obtained from the Atomic Energy Commission. Purity was insured by following the decay curve. Appropriate dilutions were analyzed for iron content, and a known aliquot as $FeCl_3$ was administered by stomach tube to fasting dogs. Procedures for determining radioactivity were essentially those as reported by Yuile *et al.* (8).

Determinations of the level of iron in the plasma were done by the method of Kitzes, Elvehjem and Schuette (9).

Dogs were rendered anemic by the subcutaneous injection of acetylphenylhydrazine dissolved in distilled water. The dose varied from time to time depending upon the level of anemia desired, but was usually between 200 and 400 mg. every other day. Care was taken to minimize hemoglobinemia in order to avoid loss of iron by renal excretion of hemoglobin.

After various periods of anemia, the dogs were given a single dose of a known amount of radioactive iron and the administration of acetylphenylhydrazine was stopped. The appearance of radioactivity in the erythrocytes was determined at weekly intervals for three weeks by which time the hematocrit readings had returned to normal. As controls most of the dogs were later given another dose of radioactive iron to determine absorption under normal conditions.

Calculation of absorption was based upon an estimation of the blood volume and the concentration of radioactivity in the packed erythrocytes. The blood volume was assumed to be 8 per cent of the body weight. This value was multiplied by the venous hematocrit to give the red cell volume. The product of the red cell volume and the concentration of radioactivity in the packed red cells yielded the total circulating radioactivity. From this the per cent of utilization was computed.

$$\% \text{ utilized} = \frac{\text{body weight} \times 0.08 \times \text{hematocrit} \times \text{radioactivity per ml. RBC} \times 100}{\text{radioactivity administered}}$$

As will be seen, the difference between anemic phenylhydrazine treated and control animals was so great that the relatively small errors involved in this calculation were negligible. The amount of radioactive iron utilized for hemoglobin synthesis was assumed to be a close estimate of the amount absorbed.

RESULTS

The results are summarized in the table. From the figures in column 3 it will be seen that all dogs had hematocrit values in the normal range at the beginning of the experiments. Hematocrit readings taken at the time the radioactive iron was given are listed in column 4. In general these values are representative of the degree of anemia induced during acetylphenylhydrazine administration, but moderate fluctuations were unavoidable. Levels of iron in the plasma are given in columns 5 and 6. All values fell within the normal range at the beginning of the experiments. Following administration of acetylphenylhydrazine the levels of iron in the plasma were in general higher than the baseline values. Dog 393 had a low plasma iron level after two weeks of acetylphenylhydrazine. We have noted in other animals that there is frequently a period, early in the course of phenylhydrazine anemia during which the level of plasma iron may be temporarily depressed. Dog 400 after prolonged phlebotomy had a low concentration of iron in the plasma. This is the expected finding.

The size in milligrams of the labelled dose of iron is given in column 7. In most of these experiments about 5 mg. were given. When larger or smaller amounts were given to anemic dogs, the controls were done with the same dose. Dog 400 received a relatively large amount, 16.6 mg., and dog 398 received a very small amount, 0.47 mg., in one experiment.

The amount of radioactivity found in the erythrocytes three weeks later is given in the last column. The figures are computed as per cent of the administered radioactivity and were calculated as described above under Methods. The figure in parentheses after dog 400 indicates the per cent of the radioactivity administered which was found by determining the amount of radioactivity removed by prolonged bleeding. This procedure withdrew from storage all the iron available for hemoglobin synthesis. This figure of 10 per cent was in good agreement with the 8.8 per cent previously estimated to be in the peripheral blood. This agreement indicated that all or nearly all of the iron absorbed from the gastrointestinal tract was utilized for hemoglobin synthesis rather than being deposited in the iron stores.

It is apparent from the figures in this column that under the experimental conditions the absorption of iron from the gastrointestinal tract was increased several fold above that of the controls. Dog 400 absorbed 10 per cent of 16.6 mg. of $FeCl_3$ after four months of anemia induced by acetylphenylhydrazine. After the dog had been rendered iron deficient by prolonged bleeding, he absorbed 13 per cent of a dose of the same size. The dog was allowed to regenerate his hemoglobin with the aid of an adequate iron intake by mouth and 0.3 Gm. of ordinary iron administered intravenously. After this he absorbed only 2.9 per cent of a 16.4 mg. dose of $FeCl_3$. Dog 398 absorbed 13 per cent of 0.47 mg. after three months of acetylphenylhydrazine and only 1.8 per cent of a test dose of the same size when the hematocrit had returned to normal. In the remaining experiments a dose of 5 mg. of $FeCl_3$ was given. After 34 days of anemia, dog 403 absorbed 38 per cent, a figure clearly above the normal absorption of 2 to 6 per cent. Two dogs were given a test dose of radioactive ferric chloride after only two weeks of anemia. Dog 393 absorbed only 4.2 per cent, whereas dog 392 absorbed 13 per cent. It seems that two weeks of anemia may be effective in some animals, but not in others.

DISCUSSION

Phenylhydrazine anemia in these dogs consistently increased gastrointestinal iron absorption if the animals were anemic for longer than 14 days. The increased iron absorption was not due to lack of iron in the body because: 1) Care was taken to prevent excessive iron loss from hemoglobinuria;

2) iron lost in the bile was probably insignificant in amount (10); and 3) the dogs recovered promptly from the anemia when phenylhydrazine administration was stopped. It seems reasonable, therefore, to attribute the excessive iron absorption to the anemia.

The explanation of this increased absorption of iron is obscure. Granick (7) has suggested that anoxia produced by anemia alters the redox level of the mucosal cells. Such a change, affecting the hypothetical ferritin mechanism of the cells, would allow increased amounts of iron to enter the body. This suggestion seems reasonable, but there is as yet little direct evidence to support it. Moreover, factors other than anemia can cause increased iron absorption. For example, the dietary siderosis produced by Hegsted, Finch, and Kinney (11) is not known to be associated with anemia. Somewhat similar diets have also caused increased absorption of iron in the face of a normal level of hemoglobin (12). Whether the intestinal mucosa is anoxic when rats are fed such deficient diets is not known, however.

Increases in mucosal iron have been shown to suppress the absorption of iron in anemic iron deficient dogs (13). There also is evidence indicating that in states of iron excess induced by diet the rate of absorption of iron is decreased (12). During the hemolytic process induced by acetylphenylhydrazine one would expect the mucosal and storage iron to be higher than normal, thus tending to decrease the absorption, rather than allowing increased absorption, but such is not the case. It would seem reasonable then to suspect that an anemia of long duration can overcome the "mucosal block" thought to be induced by the presence of iron in the mucosal cells, even though acute anemia does not do so (14).

The increased absorption of iron in these dogs took place when the concentration of iron in the plasma was elevated. This observation supports earlier work (3, 15) discounting the influence of plasma iron levels upon the absorption of iron from the gastrointestinal tract.

A difference between species seems to exist with respect to the effect of acetylphenylhydrazine upon the absorption of a single dose of iron from the gastrointestinal tract. Rats fed acetylphenylhydrazine for one month absorbed less of a single dose of an iron salt than control rats (16). The authors stated, however, that long term experiments indicated an increased absorption of iron in rats fed acetylphenylhydrazine.

Utilization for hemoglobin synthesis of orally fed iron as a measure of absorption from the intestinal tract has been criticized (3). Such criticism does not apply to these experiments since falsely low rather than falsely high values for iron absorption would have been obtained if a large fraction of recently absorbed iron had been diverted from the hemoglobin to stores. Falsely high values would have resulted only if labelled iron from stores had been incorporated into the red cell mass. This could have happened only in dogs 393 and 392 in our series, since none of the other dogs had received any radioactive iron prior to being placed on acetylphenylhydrazine. Furthermore, it has been recently demonstrated that only a small frac-

TABLE I

The absorption of iron during hemolytic anemia in dogs

Dog	Conditions	Hematocrit		Plasma iron		Dose of radioactive ferric chloride	Utilization	
		Normal	Anemic	Normal	Anemic			
		%	%	γ/100 ml.	γ/100 ml.	mg.	% of dose	
400	APH* 4 months	46.7	32.4	103	194	16.6	8.8	(10)†
400	iron deficient	50.0	37.8	103	60	16.6	13	
400	normal	48.1	—	198	—	16.4	2.9	
398	APH 3 months	52.4	34.0	104	165	.47	13	
398	normal	48.1	—	—	—	.47	1.8	
398	APH 3 months	48.0	27.6	—	248	5.42	17	
398	normal	50.0	—	—	—	5.42	2.6	
403	APH 34 days	60.0	30.2	136	210	5.01	38	
401	APH 3 weeks	53.9	34.0	159	161	5.01	25	
393	APH 2 weeks	57.1	30.0	141	70	5.01	4.2	
393	normal	43.3	—	—	—	5.01	1.8	
392	APH 2 weeks	44.1	23.0	175	225	5.01	13	

* Indicates the subcutaneous injection of acetylphenylhydrazine.
† Based upon measurement of radioactive iron removed by repeated and prolonged phlebotomy.

tion of stored iron is used to replenish the iron of hemoglobin during recovery from anemia caused by acetylphenylhydrazine (17).

The fact that absorption of iron from the gastrointestinal tract is increased during a hemolytic anemia has certain clinical implications. The use of medicinal iron in anemias not caused by a deficiency of iron is possibly dangerous. Indeed, excessive iron deposition in the viscera of a patient with hereditary sypherocytosis has occurred (18). This patient was given medicinal iron for many years as a placebo. The effects of such excessive deposits of iron are not yet clear, but it would seem wise to avoid excessive iron deposition if possible.

SUMMARY

Dogs were given subcutaneous injections of acetylphenylhydrazine to make them anemic. After various periods of anemia a test dose of radioactive iron was given by stomach tube.

All animals which had been anemic at least three weeks absorbed more iron from the gastrointestinal tract than controls. One of two dogs which were anemic for only two weeks absorbed more than a normal amount of iron.

It was concluded that hemolytic anemia caused increased absorption of iron in dogs.

REFERENCES

1. Hahn, P. F., Bale, W. F., Lawrence, E. O., and Whipple, G. H., Radioactive iron and its metabolism in anemia. Its absorption, transportation, and utilization. J. Exper. Med., 1939, **69**, 739.
2. Copp, D. H., and Greenberg, D. M., A tracer study of iron metabolism with radioactive iron. I. Methods: absorption and excretion of iron. J. Biol. Chem., 1946, **164**, 377.
3. Dubach, R., Callender, S. T. E., and Moore, C. V., Studies in iron transportation and metabolism. VI. Absorption of radioactive iron in patients with fever and with anemias of varied etiology. Blood, 1948, **3**, 526.
4. Muirhead, E. E., Crass, G., Jones, F., and Hill, J. M., Iron overload (hemosiderosis) aggravated by blood transfusions. Arch. Int. Med., 1949, **83**, 477.
5. Wyatt, J. P., Mighton, H. K., and Moragues, V., Transfusional siderosis. Am. J. Path., 1950, **26**, 883.
6. Cartwright, G. E., Wintrobe, M. M., and Humphreys, S., Studies on anemia in swine due to pyridoxine deficiency, together with data on phenylhydrazine anemia. J. Biol. Chem., 1944, **153**, 171.
7. Granick, S., Iron metabolism and hemochromatosis. Bull. New York Acad. Med., 1949, **25**, 403.
8. Yuile, C. L., Bly, C. G., Stewart, W. B., Izzo, A. J., Wells, J. C., and Whipple, G. H., Plasma and red cell radioiron following intravenous injection. Turpentine abscesses in normal and anemic dogs. J. Exper. Med., 1949, **90**, 273.
9. Kitzes, G., Elvehjem, C. A., and Schuette, H. A., The determination of blood plasma iron. J. Biol. Chem., 1944, **155**, 653.
10. Hawkins, W. B., and Hahn, P. F., Biliary excretion of radioactive iron and total iron as influenced by red cell destruction. J. Exper Med., 1944, **80**, 31.
11. Hegsted, D. M., Finch, C. A., and Kinney, T. D., The influence of diet on iron absorption. II. The interrelation of iron and phosphorus. J. Exper. Med., 1949, **90**, 147.
12. Stewart, W. B., and Shils, M. E., Unpublished observations.
13. Stewart, W. B., Yuile, C. L., Claiborne, H. A., Snowman, R. T., and Whipple, G. H., Radioiron absorption in anemic dogs. Fluctuations in the mucosal block and evidence for a gradient of absorption in the gastrointestinal tract. J. Exper. Med., 1950, **92**, 375.
14. Hahn, P. F., Bale, W. F., Ross, J. F., Balfour, W. M., and Whipple, G. H., Radioactive iron absorption by gastro-intestinal tract. Influence of anemia, anoxia, and antecedent feeding. Distribution in growing dogs. J. Exper. Med., 1943, **78**, 169.
15. Yuile, C. L., Hayden, J. W., Bush, J. A., Tesluk, H., and Stewart, W. B., Plasma iron and saturation of plasma iron-binding protein in dogs as related to the gastrointestinal absorption of radioiron. J. Exper. Med., 1950, **92**, 367.
16. Saylor, L., and Finch, C. A., Determination of iron absorption using two isotopes of iron. Am. J. Physiol., 1953, **172**, 372.
17. West, H. D., Hahn, P. F., Clark, W. F., and Chappelle, E. W., Labeled iron stores in study of iron liberated during red cell destruction by acetylphenylhydrazine. Am. J. Physiol., 1952, **169**, 194.
18. Wallerstein, R. O., and Robbins, S. L., Hemochromatosis after prolonged oral iron therapy in a patient with chronic hemolytic anemia. Am. J. Med., 1953, **14**, 256.

STUDIES ON THALASSEMIA [1, 2]

PART I. AN EXTRACORPUSCULAR DEFECT IN THALASSEMIA MAJOR

PART II. THE EFFECTS OF SPLENECTOMY IN THALASSEMIA MAJOR WITH AN ASSOCIATED ACQUIRED HEMOLYTIC ANEMIA

By HERBERT C. LICHTMAN, R. JANET WATSON, FELIX FELDMAN, VICTOR GINSBERG, AND JEAN ROBINSON

(From the Department of Medicine, State University of New York at New York City, College of Medicine, and the Kings County Hospital, Brooklyn, N. Y.)

(Submitted for publication July 8, 1953; accepted August 5, 1953)

PART I

AN EXTRACORPUSCULAR DEFECT IN THALASSEMIA MAJOR

Normal red blood cells, when transfused to patients with thalassemia, have been shown to have a normal life span (1–4). It has also been demonstrated that transfused erythrocytes from donors with thalassemia major rapidly disappear from the circulation of normal recipients (2, 4). This evidence supports the contention that the hemolytic component of the anemia in thalassemia is due to an intracorpuscular defect, as is the case in congenital spherocytic anemia (5) and in sickle cell anemia (6).

We have had the opportunity to observe repeatedly approximately 20 children with thalassemia major during the past five years. All of these subjects have required blood transfusions to alleviate such recurring symptoms as listlessness, irritability, anorexia, fatigue, palpitations, weakness or dizziness. It was evident after review of the transfusion records that many of these children were receiving 500 cc. supplements of blood, or the equivalent in packed erythrocytes, at very short intervals, *i.e.*, every ten days or every 25 days. Since (a) prior to transfusion, hemoglobin and hematocrit levels in any one patient were fairly constant (and definitely not rising over a period of several years), and since (b) there was no external loss of blood, it seemed logical to assume that the transfused blood was not surviving the expected normal span of 120 days. Other possible explanations were that each transfusion caused a decrease in endogenous blood formation or that both of these mechanisms were involved. It was the purpose of this study to test the first assumption by measuring the survival of normal fresh blood in seven recipients with thalassemia major.

METHODS

The survival of transfused erythrocytes was determined by the method of DeGowin, Sheets, and Hamilton (7). Blood from normal type O donors was collected aseptically in vacuum bottles containing "acid-citrate-dextrose" solution and was transfused to the test subjects within 36 hours. Compatibility of donor cells was assured after cross-matching by the use of the indirect Coombs test. Five of the recipients were type A and one was type AB. Anti-A sera were used in dilutions producing minimal inagglutinable cell counts. Sera capable of yielding baseline counts below 25,000 cells per cu. mm. were used. Aliquots of the selected sample of serum were kept in the deep freeze unit and were used for successive measurements during each erythrocyte life span study. One patient whose blood was type O, MN, was transfused with O, N cells. Lederle's anti-M serum was used for agglutination.

The reliability of this method in our hands was demonstrated in normals and in patients with a variety of disorders other than acquired hemolytic anemia. Survival of transfused erythrocytes for 100 to 130 days was found in agreement with results reported by others (7–10).

MATERIAL

The seven patients in this series were examples of severe thalassemia major. All were of Sicilian descent and were first noted to be anemic in infancy, when blood examinations demonstrated red blood cell abnormalities such as microcytosis, hypochromia, basophilic stippling, normoblastosis, target cells, marked anisocytosis and poikilocytosis with many small fragmented forms. The erythrocytes showed increased resistance to hypotonic saline solutions. Examination of bone marrow aspirations

[1] Presented in part at the meeting of the American Federation for Clinical Research, Eastern Section, at New York City, December, 1951.

[2] This study was supported in part by a Grant from the American Cyanamid Corporation, Lederle Laboratories Division.

TABLE I

Hematologic observations in thalassemia major before splenectomy

Case no.	Age *yrs.*	Sex	Average pre-transfusion Hb. level *Gm. %*	Average interval between 500 cc. transfusion *days*	Calculated* whole blood requirements *cc./day*	Measured red cell survival *days*	Half life of transfused red cells *days*
1. P. T.	5½	M	7.0	19.4	26.6	13	7
2. F. L.	8	F	6.0	21.4	23.3	33	8
3. C. A.	7	F	6.5	25.1	19.7	22	8½
4. L. I.	16	M	6.0	10.7	46.6	55	5½
5. D. C.	6	F	7.0	15	31	96	35
6. R. R.	9	M	7.0	19.6	23.4	10	5
7. J. C.	7	M	7.0	18	23.4	26	9

* These figures were calculated by dividing the total volume of whole blood received by transfusion during the previous one to two years by the number of days during this period.

revealed intense myeloid activity with normoblastic hyperplasia. Iron deficiency was excluded by the finding of high serum iron levels and by the failure of the anemia to respond to the administration of preparations containing iron. Splenomegaly was prominent along with moderate hepatomegaly. Each child possessed the mongoloid facial appearance so often noted in this disease (11), in addition to the characteristic roentgenographic abnormalities in the bones (12). Detailed hematologic, genetic and roentgenologic studies of these patients will be reported elsewhere.

RESULTS AND DISCUSSION

The circulating "life span" of normal red blood cells transfused to these patients was markedly shortened in all but one instance (see Table I and Figures 1–5). The "half life" of the transfused erythrocytes ranged from five to nine days in six of the patients. In Case 5, where the total survival time of the transfused red cells approximated 96 days, the "half life" of these cells was only 35 days. The rate of elimination of the cells in this patient, like those of Cases 2 and 4 (see Figure 2 and 4), is such that it appears curvilinear when charted. This has been described by Brown, Hayward, Powell, and Witts as characteristic of a random destructive mechanism which is independent of the age of the cells (13). The shortened sur-

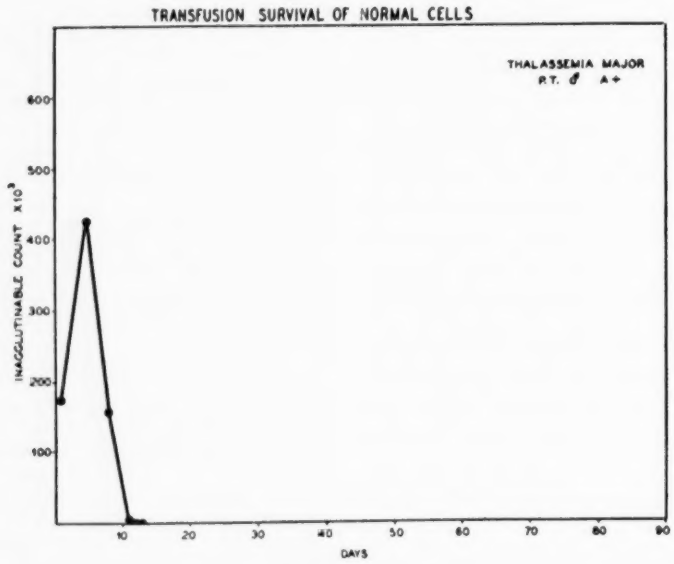


FIG. 1. CASE 1

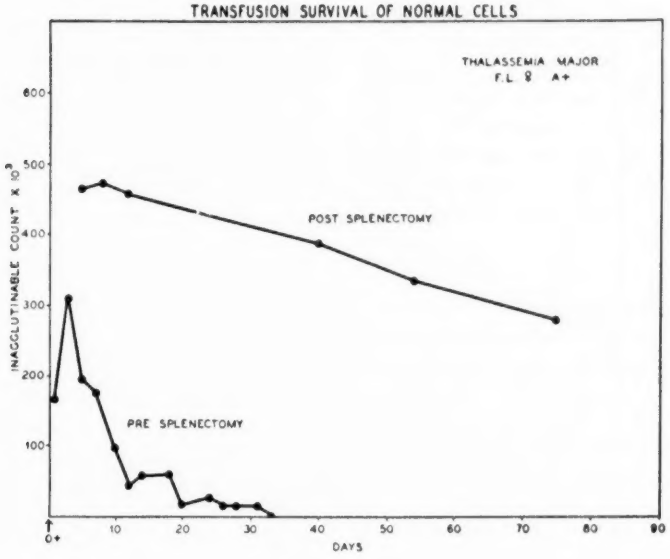


FIG. 2. CASE 2

vival of transfused red cells indicates that in all of these patients an extracorpuscular type of hemolytic anemia exists in addition to any other mechanisms operative in thalassemia major.

In four previously reported studies (1–4) on a total of seven separate patients with thalassemia the survival of normal transfused erythrocytes was found to be normal. Sufficient data are available in five of these studies to demonstrate differences in case material which may explain the discrepancy between their results and ours. Four of the previously reported patients were below two years of

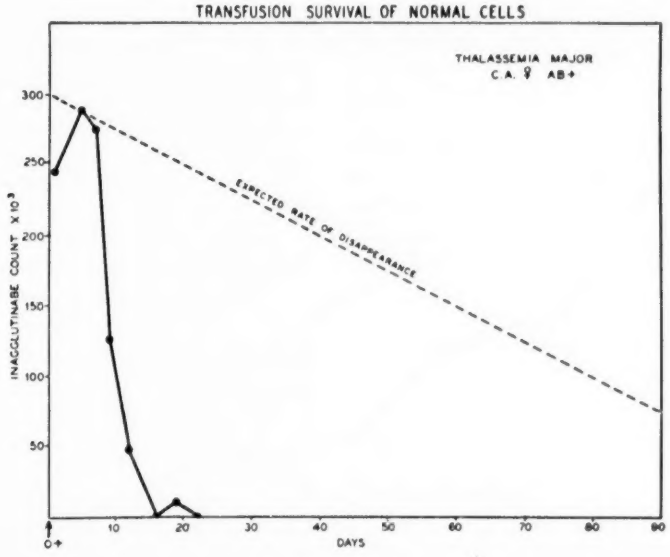


FIG. 3. CASE 3

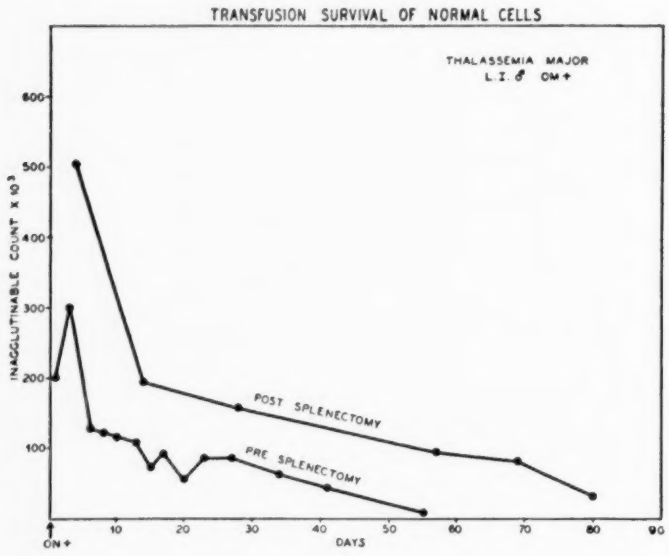


FIG. 4. CASE 4

age, and one who was eight years old had never required any blood transfusions (see Table II). The patients included in the present study were between five and 16 years old. Their records indicate that increased demands for transfusions did not become evident before the age of three.

It may be that the development of an extracorpuscular hemolytic anemia in thalassemia is dependent on an immunologic mechanism related to the large number of transfusions received by some of these patients over a period of several years. Circulating antibodies have not been demonstrated

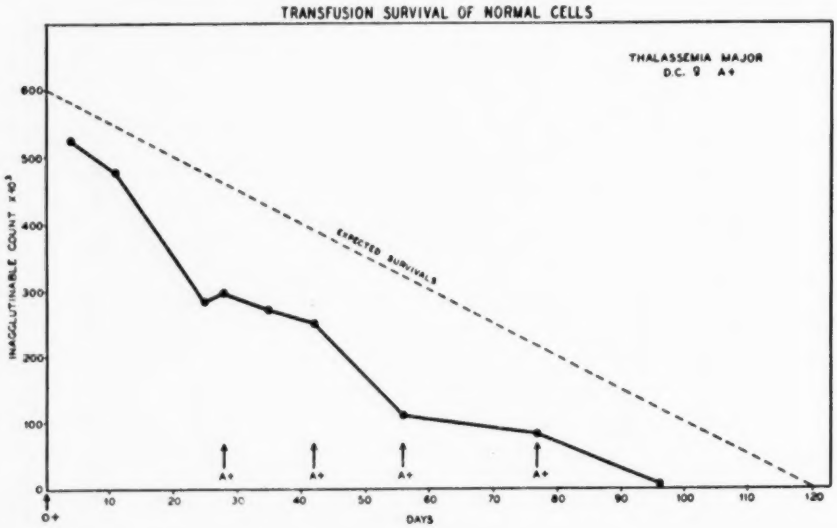


FIG. 5. CASE 5

TABLE II

Previously reported cases of thalassemia with normal erythrocyte survival of transfused cells

Case no.	Age	Frequency of transfusions	Observer
1	8 yrs.	Never transfused	Kaplan and Zuelzer (2)
2	2 yrs.	56–84 days	Kaplan and Zuelzer
3	7 wks.	Never transfused	Hamilton, Sheets, and DeGowin (1)
4	9 mos.	?	Frontali and Stegagno (4)
5	18 mos.	?	Frontali and Stegagno
6	?	?	Neber and Dameshek (3)
7	?	?	Neber and Dameshek

in our patients by the use of trypsin treated red cells (14) or by the indirect Coombs test (15). Further immunologic studies are in progress.

The persistent marked splenomegaly increasing with age suggested the possibility that the extracorpuscular hemolytic component was related to the development of an abnormal function of the spleen in these patients. In Part II of this report the role of the spleen will be assessed.

SUMMARY

1. Seven children with thalassemia major who required frequent blood transfusions in order to maintain hemoglobin levels above 6 to 7 Gm. per cent were studied with a modification of the Ashby test for survival time of transfused normal erythrocytes.

2. In each instance a shortened survival of the transfused erythrocytes was noted. This finding demonstrates that an extracorpuscular hemolytic anemia was present in these patients in addition to any other mechanisms operative in the pathogenesis of the anemia in thalassemia major.

PART II

THE EFFECTS OF SPLENECTOMY IN THALASSEMIA MAJOR WITH AN ASSOCIATED ACQUIRED HEMOLYTIC ANEMIA

Surgical removal of the spleen has been demonstrated repeatedly to be helpful in certain patients with hemolytic anemia (16–19) and curative in others (19, 20). How this procedure exerts its beneficial effect has been the subject of much investigation and speculation but a definitive explanation of this mechanism has not yet been supplied. Furthermore, in the presence of the extracorpuscular type of hemolytic anemias there are no reliable criteria available with which one can predict whether splenectomy will be of benefit.

Because of the clear evidence of the existence of an extracorpuscular hemolytic anemia in the seven patients with thalassemia major described above, splenectomy was performed in each instance. It is the purpose of this report to assess the value of the the operation in these selected cases.

RESULTS

The data on transfusion requirements of five patients who have been observed for longer than six months following splenectomy are summarized in Table III. A comparison of rates of blood replacement can also be seen in Figure 6. Transfusion requirements fell significantly during the period of observation in four instances, *i.e.*, 19 per cent, 21.3 per cent, 28.3 per cent and 36 per cent of preoperative figures. The fifth patient (Case 4) was not benefited. His requirements for blood after splenectomy were 108 per cent of preoperative levels. In the first four patients the intervals between transfusions of 500 cc. of whole blood increased from a range of 15 to 25 days before operation to a range of 57 to 119 days after splenectomy.

Actual measurements of the survival of the transfused blood were made, using the previously described modification of Ashby's test in two instances after splenectomy. As demonstrated in Figure 2, the rate of elimination of transfused erythrocytes after splenectomy was normal, in sharp contrast to the markedly shortened preoperative cell survival. This patient (Case 2) was greatly improved following splenectomy in that her transfusion interval increased from 21 days to 75.0 days.

In Case 4 (Figure 4) the half life of transfused red cells is seen to be only slightly increased, *i.e.*,

TABLE III

Post splenectomy—calculated whole blood requirements

Case no.	Average interval between 500 cc. transfusions	Calculated whole blood requirements	Fraction of pre-operative transfusion rates	Period of observation post-operatively
	days	*cc./day*	%	*months*
1. P. T.	57.7	8.6	36.0	12
2. F. L.	75.0	6.6	28.3	12
3. C. A.	119.0	4.2	21.3	12
4. L. I.	9.8	50.5	108.	9
5. D. C.	84.0	5.9	19.0	6

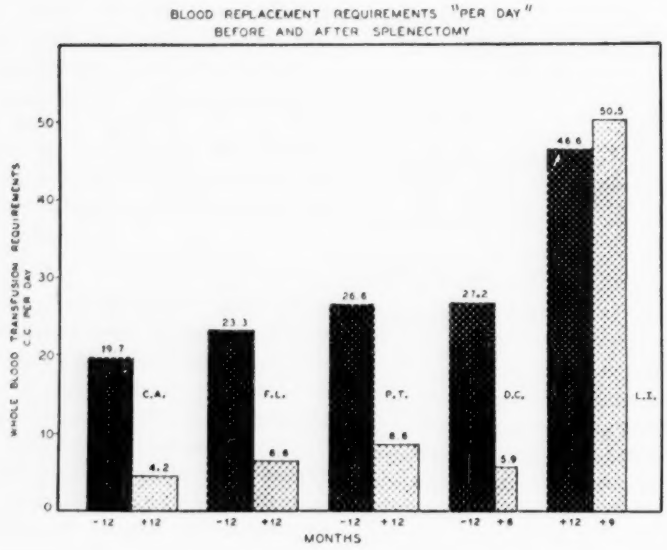


FIG. 6

14 days as compared to five and one half days. This was the single patient studied whose transfusion requirements were not improved by splenectomy.

DISCUSSION

Splenectomy has been performed in thalassemia major many times in the past. Some (21, 22) have recommended the operation for its beneficial hematological effects. Most observers feel that splenectomy is productive of little clinical improvement apart from the mechanical relief gained by removal of a large and heavy organ from the persistently distended abdomen (23, 24).

The present study indicates that in a select group of patients, splenectomy is of value in most instances. The selection in this series depended on the findings of an extracorpuscular type of hemolytic anemia. This was demonstrated by red cell survival measurements. It is our impression that the chronic pronounced splenomegaly, persisting for at least three years, was instrumental in causing a rapid destruction of normal transfused blood cells. That the spleen may not be the only organ involved in this pathological capacity is suggested by a lack of improvement in Case 4 following operation. It is of interest that in the entire series this patient had the greatest degree of hepatic enlargement.

An extracorpuscular hemolytic anemia can be predicted with relative confidence when: 1) Transfusions are required to maintain adequate hemoglobin levels at intervals shorter than the normal survival of transfused blood; and when 2) there is no other cause for a rapid fall in circulating hemoglobin concentration such as bleeding or the expansion of the intravascular space, or a temporary decrease in endogenous erythrocyte production. That variations in endogenous erythrocyte production do occur in thalassemia has been demonstrated (1). Just how important this factor is in affecting the levels of circulating hemoglobin in thalassemia awaits further study. It is tempting to postulate that the discrepancies between the measured red cell survival and the required interval between blood transfusions are related to fluctuations in endogenous erythrocyte production. This is suggested in Case 5 (Figure 5). A transfused red cell survival of 96 days with a "half life" of 35 days was found. In spite of this however, administration of 500 cc. of blood was required at intervals of 15 days in order to keep the circulating hemoglobin concentration above 7 Gm. per cent.

The number of circulating erythrocytes present in the body at any specific moment is a function of four variables: 1) The amount of blood transfused; 2) the rate of destruction of these cells; 3) the rate of endogenous production of erythrocytes; and 4) the rate of destruction of these endogenously produced cells. In this study we have data on the first two variables. Knowledge of the dynamics of factors 3) and 4) is necessary for a more complete understanding of the mechanism of the anemia in these patients.

SUMMARY

1. Five patients with thalassemia major in whom an extracorpuscular type of hemolytic anemia was demonstrated, were subjected to surgical removal of their spleens.

2. The requirements of four of these patients for supportive blood transfusions were markedly reduced. One patient was unchanged clinically.

3. The survival of transfused erythrocytes was measured in two instances after splenectomy. In one instance the transfused erythrocytes were found to have a normal life span after operation as compared to a total survival of 33 days before splenectomy. In the second patient who was not benefited by splenectomy there was no significant change in the survival of transfused erythrocytes.

REFERENCES

1. Hamilton, H. E., Sheets, R. F., and DeGowin, E. L., Studies with inagglutinable erythrocyte counts. II. Analysis of mechanism of Cooley's anemia. J. Clin. Invest., 1950, **29**, 714.
2. Kaplan, E., and Zuelzer, W. W., Erythrocyte survival studies in childhood. II. Studies in Mediterranean anemia. J. Lab. & Clin. Med., 1950, **36**, 517.
3. Neber, J., and Dameshek, W., The improved demonstration of circulating antibodies in hemolytic anemia by the use of a bovine albumin medium. Blood, 1947, **2**, 371.
4. Frontali, G., and Stegagno, G. A., The life span of red cells in Mediterranean anemia. Comptes rendus du troisième congrès de la Société International Européenne d'Hématologie. Edizioni Mediche E Scientifiche, Rome, 1952, 545.
5. Dacie, J. V., and Mollison, P. L., Survival of normal erythrocytes after transfusion to patients with familial hemolytic anemia (acholuric jaundice). Lancet, 1943, **1**, 550.
6. Callender, S. T. E., Nickel, J. F., and Moore, C. V., Sickle cell disease: studied by measuring the survival of transfused red blood cells. J. Lab. & Clin. Med., 1949, **34**, 90.
7. DeGowin, E. L., Sheets, R. F., and Hamilton, H. E., Studies with inagglutinable erythrocyte counts. I. A method for measurement of net gain or deficit of red cells in the human subject. J. Clin. Invest., 1950, **29**, 693.
8. Mollison, P. L., The survival of transfused erythrocytes, with special reference to cases of acquired haemolytic anaemia. Clin. Sc., 1947, **6**, 137.
9. Ashby, W., The determination of the length of life of transfused blood corpuscles in man. J. Exper. Med., 1919, **29**, 267.
10. Callender, S. T., Powell, E. O., and Witts, L. J., The life span of the red cell in man. J. Path. & Bact., 1945, **57**, 129.
11. Wintrobe, M. M., Clinical Hematology, ed. 3, Philadelphia, Lea & Febiger, 1951, 684.
12. Caffey, J., The skeletal changes in the chronic hemolytic anemias (erythroblastic anemia, sickle cell anemia and chronic hemolytic icterus). Am. J. Roentgenol., 1937, **37**, 293.
13. Brown, G. M., Hayward, O. C., Powell, E. O., and Witts, L. J., The destruction of transfused erythrocytes in anaemia. J. Path. & Bact., 1944, **56**, 81.
14. Morton, J. A., and Pickles, M. M., The use of trypsin in the detection of incomplete anti-Rh antibodies. Nature, 1947, **159**, 779.
15. Coombs, R. R. A., Mourant, A. E., and Race, R. R., Detection of weak and "incomplete" Rh agglutinins: a new test. Lancet, 1945, **2**, 15.
16. Dameshek, W., and Schwartz, S. O., Acute hemolytic anemia (acquired hemolytic icterus, acute type). Medicine, 1940, **19**, 231.
17. Stats, D., Wasserman, L. R., and Rosenthal, N., Hemolytic anemia with hemoglobinuria. Am. J. Clin. Path., 1948, **18**, 757.
18. Stickney, J. M., and Heck, F. J., Primary nonfamilial hemolytic anemia. Blood, 1948, **3**, 431.
19. Welch, C. S., and Dameshek, W., Splenectomy in blood dyscrasias. New England J. Med., 1950, **242**, 601.
20. Doan, C. A., Curtis, G. M., and Wiseman, B. K., The hemolytopoietic equilibrium and emergency splenectomy. J. A. M. A., 1935, **105**, 1567.
21. Govan, C. D., Erythroblastic anemia of Cooley, observations on the effect of splenectomy performed on identical twins. J. Pediat., 1946, **29**, 504.
22. Shapiro, I., Schneck, H., and Etess, A. D., Splenectomy in two Chinese siblings with Mediterranean anemia. New York State J. Med., 1952, **52**, 1426.
23. Wolman, I. J., and Dickstein, B., Changing concepts in Mediterranean (Cooley's) anemia. Am. J. M. Sc., 1946, **212**, 723.
24. Baty, J. M., Blackfan, K. D., and Diamond, L. K., Blood studies in infants and in children. I. Erythroblastic anemia; a clinical and pathologic study. Am. J. Dis. Child., 1932, **43**, 667.

ADRENAL CORTICAL REGULATION OF THE DISTRIBUTION OF WATER AND ELECTROLYTES IN THE HUMAN BODY[1]

By A. G. HILLS, T. M. CHALMERS,[2] G. D. WEBSTER, JR.,[3] AND OTTO ROSENTHAL
WITH THE TECHNICAL ASSISTANCE OF HANA CONOVER AND ELIZABETH SONGSTER

(From the Edward B. Robinette Foundation, Medical Clinic, and the Chemical Section, Department of Medicine, Hospital of the University of Pennsylvania, and the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, Pa.)

(Submitted for publication November 10, 1952; accepted August 11, 1953)

Marked disturbances of fluid and electrolyte distribution have been reported to accompany experimental adrenal insufficiency. Swingle and his associates (1–4), studying the development of acute adrenal insufficiency in dogs, noted that the external loss of sodium and water appeared to be quantitatively inadequate to account for the observed dehydration and hemoconcentration. They concluded that adrenal cortical hormone renders intracellular water and electrolytes available for replenishment of the depleted extracellular fluid in adrenal insufficiency (3, 4). More recently, other studies in dogs have shown that a large decrease of the inulin space follows adrenalectomy (5), and that the calculated loss of sodium and chloride from the mannitol and thiocyanate spaces during acute adrenal insufficiency consistently exceeds net external loss (6). Internal transfers of water and electrolytes are therefore indicated.

Analyses of the soft tissues of animals of various species dying in acute adrenal insufficiency have, however, been consistently reported to indicate either no change or an actual decrease of cellular sodium in all soft tissues studied, although cell water and potassium are said to be increased (7–11). The intracellular migration of water has generally been assumed to be a passive osmotic process resulting from extracellular hyponatremia (8, 9, 10). Bone has been suggested as a possible site for the sodium transferred from the mannitol space, but relevant evidence is lacking (6, 12). The subject has recently been reviewed by Overman (13).

In this communication are reported studies of the effects of acute adrenal insufficiency and of adrenal steroids upon fluid and electrolyte distribution in man.

MATERIALS AND METHODS

I. *Clinical material and design of experiments*

Eleven patients previously subjected to subtotal adrenalectomy because of severe hypertensive disease and one patient with hypertensive disease not surgically treated served as experimental subjects. The experiments were of three kinds: 1) Observations of effects of acute adrenal insufficiency experimentally provoked by a standard regimen; 2) control observations in two of the subtotally adrenalectomized patients who did not develop adrenal insufficiency on the standard regimen and in one unoperated hypertensive individual subjected to drastic reduction of sodium intake; and 3) studies of the effects of various steroids upon fluid distribution in certain of the adrenal-deficient patients.

Nine of the patients who had previously undergone subtotal adrenalectomy were subjected to a standard regimen designed to induce a state of acute adrenal cortical insufficiency. The procedure was as follows: patients were admitted to the Metabolic Unit for a six-day balance study, which was subdivided into a three-day control period followed by a three-day experimental period. The diet and fluid intake were kept constant throughout the study, save for a substantial supplement of sodium and chloride given to five patients during the control period. Each patient continued during the control period to receive his usual steroid replacement therapy (Table I).

During the subsequent three-day experimental period all steroid therapy was withdrawn, and the constant daily diet received by all patients was of standard composition in all respects, including its content of sodium (103 to 118 mEq.) and potassium (75 to 80 mEq.). Daily measurements were made of serum sodium, chloride, potassium, protein, and urea; of total urinary sodium, chloride, potassium, creatinine, and nitrogen; and of total sodium, chloride, potassium, and nitrogen in the stool and vomitus, if any.

The inulin space was measured on the morning of the last control day and again in all patients on the morning of

[1] This investigation was supported in part by research grants from the National Heart Institute, U. S. Public Health Service, the Squibb Institute for Medical Research, Sandoz Pharmaceuticals, Eli Lilly and Company, Ciba Pharmaceutical Products, Inc., and the Francis and Marian Boyer Fund.

[2] Nuffield Foundation Traveling Fellow. Present address: Medical Unit, Royal Infirmary, Cardiff, Wales.

[3] Trainee of the National Heart Institute.

TABLE I

Patients studied—description and experimental conditions

Patient	Age	Sex	Race	Surface area m^2	Daily intake during control period: Cortisone (oral) *mg.*	DCA (buccal) *mg.*	Sodium *mEq.*
I. Adrenal insufficiency (Tables II–V)							
H. L.	51	M	W	1.80	9	0	116
B. G.*	44	F	W	1.52	8	0	118
M. R.‡*	45	F	W	1.71	25	1	212
J. C.*	41	F	W	1.47	37.5	2	163
S. W.	49	M	C	1.98	25	2	282
T. S.†	36	M	W	2.04	25	4	253
M. H.*	30	F	W	1.65	37.5	0	249
II. Controls (Table VI)							
M. M.	49	F	W	1.75	4	0	175
E. M.	37	M	W	2.01	0	0	248
F. N.	40	M	W	1.74	0	0	146
III. Effect of steroids (Table VII)							
J. V.	41	M	W	1.80			
S. C.	33	F	C	1.51			

* Effect of steroid studied following precipitation of adrenal insufficiency. (Table VII).
† Steroid effect studied in separate experiment.
‡ DCA continued throughout experimental period.

the third day after hormone withdrawal. In three of these patients (B. G., M. R., and J. C.) the immediate effect upon the inulin space of intravenous cortisone, administered over a period of an hour or less, was studied at the time of the second inulin space measurement. In S. C., a subtotally adrenalectomized patient maintained in good health by sodium chloride without steroid therapy, a measurement of the effect of intravenous cortisone upon the inulin space was made without first attempting to induce adrenal cortical insufficiency by salt withdrawal (Table VII).

The balance studies were carried out on an air-conditioned metabolic ward, the room temperature being kept between 70 and 75° F. The diets were specially prepared in the Metabolic Unit from meats, canned fruits, and vegetables bought in uniform lots. The mineral and nitrogen composition of the diets was calculated from mean values of multiple analyses performed on representative aliquots.

Pertinent clinical information concerning the patients studied is summarized in Table I. Seven adrenal-deficient patients placed on the standard regimen developed symptomatic and chemical evidence of mild to severe acute adrenal insufficiency by the end of the third day of hormone withdrawal (Tables II to V inclusive). All showed little or no response, as judged by the electrolyte balances and urinary steroid excretion, to an eight-hour intravenous infusion of 10 units of corticotrophin.[4]

Two patients who had had a prior subtotal adrenalectomy (M. M. and E. M.) failed to develop symptomatic or chemical evidence of adrenal insufficiency on the standard regimen, and an eight-hour intravenous infusion of 10 units of corticotrophin was followed by clear-cut evidence of available adrenocortical reserve in terms of electrolyte balances and urinary steroid excretion. These data are presented in detail elsewhere (14). These patients, together with F. N., a hypertensive individual subjected to three days of drastic dietary salt restriction, provide control observations for comparison with the series of patients in whom acute adrenal insufficiency developed (Tables I and VI).

The effect of certain adrenal steroids on fluid distribution was examined in seven adrenal-deficient subjects. In addition to the four experiments in which immediate effects of cortisone were studied, the effect of hydrocortisone in adrenal insufficiency was observed in M. H., the effect of substitution of cortisone for desoxycorticosterone in T. S., and the effect of adding desoxycorticosterone to a basal ration of cortisone in J. V. The experimental conditions and results of these studies are presented in Table VII.

II. *Measurements and calculations*

Measurements were made of the volume of inulin distribution, after Schwartz, Schachter, and Freinkel (15), by giving an intravenous priming injection of 2.5 Gm. of inulin followed by an intravenous infusion of the major portion of a solution of 7.5 Gm. inulin in 575 ml. 5 per cent dextrose at a rate of 1 ml. per minute. After four and one-half hours, the bladder was washed out and catheter urine collections begun for three 20 to 30 minute periods. Blood samples were collected in the middle of the periods for inulin analysis, and the inulin clearance was measured. Urine was collected for approximately 18 hours after the termination of the inulin infusion. From the total inulin recovered in this sample and the constant plasma inulin, an "uncorrected" inulin space can be calculated.

[4] We are grateful to Dr. E. G. Vonder Heide, of Parke-Davis and Company, for a generous supply of this material.

In order to minimize the dead-space error, a "corrected" inulin space has been calculated, based upon an arbitrarily assumed value of 30 ml. for the volume of the urinary passages from glomerulus to bladder:

$$E_{INcr} = \frac{In_u - .03 \times (In)_u}{(In)_{sw}}$$

where

E_{INcr} = corrected inulin space in liters

In_u = total inulin in milligrams recovered in the urine after stopping the infusion

$(In)_u$ = concentration of inulin in the urine collected at the time infusion stopped in milligrams per liter

$(In)_{sw}$ = concentration of inulin in serum water during the clearance periods in milligrams per liter

In the experiments in which the immediate effect of cortisone was studied, three extra clearance periods were performed while intravenous cortisone was being infused. The inulin space, as affected by cortisone, was calculated from the total inulin recovered and the plasma inulin during the cortisone infusion. The inulin present in the body just prior to cortisone administration was obtained from the total inulin recovered by deducting the inulin infused, and adding the inulin recovered in the urine, during the cortisone infusion. The inulin space prior to the administration of cortisone was then calculated from the plasma values prior to the cortisone infusion. The correction for the pre-cortisone inulin space was based upon the urinary inulin concentration in the last pre-cortisone clearance period.

All values for inulin concentration in body water, as well as for electrolyte concentrations, were calculated from the serum concentrations and the formula for serum water of Eisenman, Mackenzie, and Peters (16):

$$(H_2O)_s = 0.986 - 0.00745P$$

where P is serum protein in grams per 100 milliliters and $(H_2O)_s$ is serum water in kilograms per liter of serum.

Values for electrolyte in the inulin space were obtained as follows: the interstitial water concentrations were calculated as plasma water values to which, in the case of sodium and chloride, the Donnan factor correction (0.95) was applied. The extracellular water concentrations of all electrolytes were calculated as follows:

$$(Cl)_{ecw} = \frac{(Cl)_{sw} + 3(Cl)_{isw}}{4}$$

where

ecw = extracellular water

sw = serum water

and

isw = interstitial water

TABLE II

Effect of acute adrenal insufficiency—serum values and inulin space measurements before and two days after hormone withdrawal in adrenal-deficient patients

Patient	Serum concentrations*					Concentration in extracellular water*			Inulin space			Inulin clearance*
	Protein Gm. %	Na mEq./L.	Cl mEq./L.	K mEq./L.	Urea N† mg. %	Na mEq./L.	Cl mEq./L.	K mEq./L.	E_{IN}* liters	ΔE_{IN} liters	ΔE_{IN} %	±S.E.M. ml./min.
H. L.	7.3	134	97	5.2	43	138.4	108.2	5.60	8.6			41 and 36
	7.2	121	98	5.5	43	124.9	99.3	5.90	7.4	−1.2	−14	40 ± 5.5
B. G.	7.6	135	99	4.8	22	139.8	110.8	5.16	9.6			99 ± 24.1
	7.8	137	99	4.6	23	142.0	110.8	4.95	7.9	−1.5	−16	100 ± 7.2
M. R.	7.3	141	98	4.2	18	145.6	109.4	4.72	7.5			77 ± 1.0
	7.5	139	98	5.2	21	143.4	109.5	5.81	5.5	−2.0	−27	97 ± 8.2
J. C.	6.8	142	104	4.2	32	145.9	115.6	4.49	7.5			50 ± 1.7
	7.1	138	104	4.2	41	142.4	115.9	4.50	4.8	−2.7	−36	45 ± 2.0
S. W.	6.9	139	107	5.3	36	143.2	119.1	5.67	15.3‡			x
	7.1	138	103	5.7	47	142.4	114.8	6.11	10.0	−5.3	−35	74 ± 16.9
T. S.	6.8	142	106	5.0	33	146.2	117.4	5.29	9.9§			x
	6.4	140	103	5.4	48	144.0	114.9	5.73	6.9§	−3.0	−30	x
M. H.	7.8	136	95	5.3	30	141.1	106.6	5.71	9.2			64 ± 5.4
	7.6	130	89	5.5	32	134.7	99.6	5.92	6.9	−2.3	−25	48 ± 7.2
										Mean	−26.1	
										S.E.M.	3.25	
										t	8.0	
										P	<.001	

* Experimental values appear below their controls.

† Serum urea calculated from blood urea, hemoglobin, and serum protein in H. L., B. G. and M. R.; measured in the other patients.

‡ Clearance not obtained. Estimated dead space correction based on urine flow.

§ Clearance not obtained. Estimated dead space correction equivalent to 0.5 liter inulin space deducted from both determinations.

TABLE III

External balances and calculated net internal transfers from the inulin space in acute adrenal insufficiency

Patient	Total electrolyte in the inulin space*						External balance					Transferred out of the inulin space				
	Na_{IN} mEq.	ΔNa_{IN} mEq.	Cl_{IN} mEq.	ΔCl_{IN} mEq.	K_{IN} mEq.	ΔK_{IN} mEq.	b_{Na} mEq.	b_{Cl} mEq.	b_K mEq.	b_N mEq.	$b_{K'}$ mEq.	$b_{Na} - \Delta Na_{IN}$ mEq.	$b_{Cl} - \Delta Cl_{IN}$ mEq.	$b_K - \Delta K_{IN}$ mEq.	$b_{K'} - \Delta K_{IN}$ mEq.	Na − Cl mEq.
H. L.	1190		930		48											
	925	−265	734	−196	44	− 4	− 69	− 52	+28	− 7.7	+46	196	144	32	50	62
B. G.	1342		1063		50											
	1122	−220	876	−187	39	−11	− 22	− 33	+13	+ 2.0	+ 8	198	154	24	19	44
M. R.	1093		820		35											
	788	−305	602	−218	32	− 3	−115	− 90	+29	− 3.7	+38	190	128	32	41	52
J. C.	1094		867		34											
	683	−411	556	−311	22	−12	−139	− 66	+18	+ 1.7	+14	272	245	30	26	27
S. W.	2191		1822		87											
	1424	−767	1148	−674	61	−26	−208	−149	+35	+ 4.9	+24	559	525	61	50	34
T. S.	1447		1162		52											
	994	−453	793	−369	40	−12	−137	−164	+51	+ 3.2	+43	316	205	63	55	91
M. H.	1298		981		53											
	929	−369	687	−294	41	−12	−119	−178	+19	− 3.5	+27	250	116	31	39	134
						Mean	−116	−105				Mean	283.0	216.7	39.0	40.0	63.4
												S.E.M.	49.3	54.2	6.03	5.03	14.2
												t	5.79	4.00	6.50	7.94	4.45
												P	<.01	<.01	<.001	<.001	<.01

* Experimental values appear below their controls.

TABLE IV

Calculated change of chloride space and calculated net internal transfers of electrolytes from the chloride space in acute adrenal insufficiency

Patient	Change			Transferred out of chloride space	
	ΔE_{Cl}* *liters*	ΔNa_{Cl}† *mEq.*	ΔK_{Cl}‡ *mEq.*	$b_{Na} - \Delta Na_{Cl}$§ *mEq.*	$b_{K'} - \Delta K_{Cl}$ *mEq.*
H. L.	+0.2	− 90	+4	21	42
B. G.	−0.3	− 21	−4	− 1	12
M. R.	−0.9	−147	+3	32	35
J. C.	−0.7	−126	−3	−13	17
S. W.	−0.8	−141	0	−67	24
T. S.	−1.2	−193	−2	56	45
M. H.	−1.1	−207	−5	88	32
Mean	−.69			17	29.5
S.E.M.	.184				4.69
t	3.75				6.31
P	<.01			Non-significant	<.001

* Calculated change of chloride space.
† Total sodium in the chloride space.
‡ Total potassium in the chloride space.
§ Minus values indicate transfers into chloride space.

on the assumption that the serum water represented 25 per cent of the total extracellular water. Electrolyte in the inulin space (Table III) is the product of the inulin space in liters times the concentration of electrolyte in extracellular water in milliequivalents per liter. The change in chloride space (ΔE_{Cl}) was calculated from the chloride balance and extracellular chloride concentration (17) on the assumption of an initial chloride space equal to the initial measured inulin space:

$$E_{Cl_2} = \frac{E_{IN_1}(Cl)_{ecw_1} + b_{Cl}}{(Cl)_{ecw_2}}$$

$$\Delta E_{Cl} = E_{Cl_2} - E_{IN_1}$$

where

E_{Cl_2} = final chloride space in liters

E_{IN_1} = initial inulin space in liters

$(Cl)_{ecw_1}$ = initial extracellular water chloride concentration in milliequivalents per liter

$(Cl)_{ecw_2}$ = final chloride concentration in extracellular water in milliequivalents per liter

b_{Cl} = external balance of chloride in milliequivalents

The water balance was calculated, with one exception, according to the formula of Lavietes (18):

$$\Delta W = W_i + (S_e - S_i) + C_i + F_b + 3.38\,N_u$$

where S_e is solids of the excreta, S_i solids of the ingesta, C_i carbohydrate ingested, F_b fat burned, and N_u urine nitrogen, all in grams.

Solids of the stool, fluid intake, and solid food were measured by desiccation to constant weight in an oven at 100° C. Urine solids were calculated by Long's coefficient (19):

Urine solids (Gm.) = 2.66 × last two figures of urinometer reading × urine volume in liters.

The fat burned was calculated according to the formula of Lavietes (18):

$$F = \frac{IL - 2.12\,C_i - 10.6\,N_u}{3.78}$$

where IL is the insensible loss of weight.

"Electrolyte unaccounted for" represents the algebraic difference between the change in the product of inulin space times extracellular water concentration of the respective electrolytes, and the net loss or gain of the corresponding electrolyte, measured as the external balance. The balances were calculated from 7 A.M. to 7 A.M. of the days on which the inulin space was measured, and the extracellular water concentration was calculated from 7 A.M. plasma values on those days. K′ represents potassium moving independently of the catabolism or anabolism of tissues, and is derived from the potassium and nitrogen balances:

$$b_{K'} = b_K - (2.4\,b_N)$$

where

b_K = potassium balance in milliequivalents

and

b_N = nitrogen balance in grams (17)

The statistical methods employed are described by Snedecor (20).

III. *Chemical methods.*[5]

Inulin determinations were made by the method of Harrison (21). Sodium and potassium were determined on a flame photometer with internal lithium standard (22). The following methods were used for the other chemical analyses: chloride, Eisenman's modification (23) of the Van Slyke method; creatinine, Bonsnes and Taussky (24); urea, Karr (25); urine nitrogen and (in some instances)

[5] We wish to thank Dr. John G. Reinhold and Dr. J. Kapp Clark for the performance of some of these analyses in their laboratories.

TABLE V

Net internal transfers of water out of inulin space and chloride space in acute adrenal insufficiency

Patient	Body weight (control) Kg.	Δ Wt. Kg.	ΔW* liters	ΔW − ΔE_{IN}† liters	ΔW − ΔE_{Cl}‡ liters
H. L.	64.88	+ .60	+.48	1.7	.28
B. G.	57.03	+ .07	+.16	1.7	.46
M. R.	71.46	− .40	−.40§	1.6	.50
J. C.	53.03	−1.28	−.90	1.8	−.20‖
S. W.	88.24	− .36	0	5.3	.80
T. S.	84.48	− .96	−.57	2.4	.63
M. H.	43.93	− .80	−.46	1.8	.64
Mean		− .45	−.24	2.33	.447
S.E.M.				.505	.124
t		Non-	Non-	4.6	3.58
P		significant	significant	<.01	<.02

* ΔW = Calculated change of total body water.
† ΔW − ΔE_{IN} = Net transfer of water out of the inulin space.
‡ ΔW − ΔE_{Cl} = Net transfer of water out of the chloride space.
§ ΔW = ΔWt. (Stool solids not measured.)
‖ Minus values indicate net transfer into the chloride space.

serum protein by a micro-Kjeldahl method using a selenium catalyst and boric acid titration; serum protein in other instances by the method of Wolfson, Cohn, Calvary, and Ichiba (26).

RESULTS

Direct observations, derived data, and interpretive calculations are presented in Tables II to VIII inclusive.

A. Effects of acute adrenal insufficiency

A large decrease in the volume of distribution of inulin was uniformly observed, amounting to 26.1 ± 3.25 per cent (Table II).[6] Net external water loss, when it occurred, was never large enough to account for the decrease of the inulin space (Table V). Ignoring certain reservations to be discussed one may regard the change of the inulin space corrected for external water balance ($\Delta W - \Delta E_{IN}$, Table V) as a measure of water transferred in adrenal insufficiency, *i.e.*, disappearing from the inulin space into some other compartment of body water.

Concentrations of electrolytes and urea nitrogen in serum and extracellular water on the day of the inulin space determinations are given in Table II, and in Table III are shown calculated total sodium, chloride, and potassium in the inulin space at the time of each measurement (Na_{IN}, Cl_{IN}, K_{IN}, Table III). Calculated net change in these quantities in association with acute adrenal insufficiency is also shown in Table III, and this change is compared with net external losses of sodium and chloride and the net external gain of potassium over the same period (b_{Na}, b_{Cl}, b_K, $b_{K'}$). Electrolyte "transferred" again represents the calculated change of electrolyte in the inulin space corrected for external balance.

A lesser but still significant calculated decrease of the chloride space was observed (ΔE_{Cl}, Table IV). Calculated change of total sodium and potassium in the chloride space is also shown (ΔNa_{Cl}, ΔK_{Cl}, Table IV). Significant "transfers" from the chloride space, calculated exactly as in the case of the inulin space, are indicated for water ($\Delta W - \Delta E_{Cl}$, Table V) and for potassium ($b_{K'}$ −

[6] If, during the precipitation of acute adrenal insufficiency, there occurred a reduction of inulin clearance to such an extent that the recovery of inulin in the 18-hour urine collection was notably less during this second measurement of the inulin space as compared with the control, a factitial decrease of the inulin space would result. To evaluate this possible source of error, inulin recovery was separately determined in two of the subjects in whom inulin clearance was in the range of the lowest values observed in the group. In these two patients (T. S. and J. C.) recoveries of 94 per cent and 93 per cent were obtained at 18 hours with inulin clearances of 47 ± 1 and 67 ± 1 ml. per min., respectively. Even a decrease from normal to half the normal filtration rate or less between measurements would therefore occasion an error of only about 7 per cent. However, the control values themselves (Table II) were already markedly subnormal—somewhat, though not strikingly, lower than is expected in Addison's disease (27, 28); and since the decreases of filtration rate in adrenal insufficiency were never large (Table II), it is difficult to suppose that a factitial decrease of the inulin space could have amounted to more than 1 or 2 per cent in these experiments.

ΔK_{Cl}, Table IV), but not for sodium ($b_{Na} - \Delta Na_{Cl}$, Table IV).

B. Control observations

In Table VI are presented data indicating that reduction of dietary salt in the manner employed in the standard regimen, with or without withdrawal of cortisone, is not followed by decrease of the inulin space when adrenal insufficiency does not ensue owing to the availability of adrenocortical reserve; and further that extreme reduction of dietary sodium did not in three days result in diminution of the inulin space in an individual possessing adequate adrenocortical function.

C. Effects of steroids

The data in Table VII indicate that cortisone, hydrocortisone, and desoxycorticosterone are all capable of effecting changes in fluid distribution in a fashion contrary to the effects of acute adrenal insufficiency. Thus, the substitution of cortisone (75 mg. daily) for desoxycorticosterone (6 mg. daily) produced in T. S. a small increase in the inulin space; the addition of desoxycorticosterone to cortisone a larger increase in J. V.; whereas a very large increase of the inulin space was observed when hydrocortisone was administered to M. H. at a time when the patient was in a state of acute adrenal insufficiency. Smaller increases in the chloride space were observed in these three patients. In two out of three patients (B. G., M. R., and J. C.) who were in a state of acute adrenal insufficiency increases of the inulin space were observed in less than one hour following administration of cortisone intravenously.

DISCUSSION

The observations reported in this communication show that external water loss is neither a conspicuous nor a constant feature of acute adrenal insufficiency in man. Internal transfers of water are, however, regularly indicated; and external loss of sodium and chloride appears to be overshadowed by the internal transfers of these ions which accompany the condition. The measurements also disclose that such internal transfers regularly precede the development of hyponatremia, for in five patients no abnormal decrease of serum sodium concentration had supervened at the time of the second (experimental) measurements. Therefore, while it is presumably true that hyponatremia of the extracellular fluid, when it occurs, will occasion osmotic transfer of water into cells, hyponatremia clearly was not the important initiator of the transfers observed in these patients.

On the other hand hyponatremia cannot be regarded as a simple consequence of the fluid transfers, since, on any interpretation of the data, more water than sodium is transferred out of the extracellular compartment. Net external loss of sodium is, however, relatively much greater than net external water loss (b_{Na}, Table III; ΔW, Table V).

TABLE VI

Salt and hormone withdrawal without resulting adrenal insufficiency—inulin space measurements

Patient	Diagnosis	Control: Daily ration: Cortisone *mg.*	Control: Daily ration: Dietary sodium *mEq.*	Experimental: Daily ration: Cortisone *mg.*	Experimental: Daily ration: Dietary sodium *mEq.*	Duration *days*	Inulin space*: E_{IN} *liters*	Inulin space*: ΔE_{IN} *liters*
M. M.	Hypertension; prior subtotal adrenalectomy; positive ACTH response	4	175	0	108	2	8.6 9.1	+0.5
E. M.	Hypertension; prior subtotal adrenalectomy; positive ACTH response	0	248	0	111	2	10.4 10.3	−0.1
F. N.	Hypertension; no surgical treatment	0	146	0	12	3	10.0 11.5	+1.5

* Experimental values appear below their controls.

TABLE VII

Effect of various steroids on fluid distribution

Patient	Control period				Experimental period				Change of chloride and inulin spaces			Concentration in extracellular water*			Electrolyte in the inulin space*						Balances			Net transfer into inulin space†		
	Duration *days*	Daily oral cortisone *mg.*	Daily buccal DCA *mg.*	Daily dietary sodium *mEq.*	Duration	Cortisone *mg.*	Buccal DCA *mg.*	Daily dietary sodium *mEq.*	ΔE_{Cl} *liters*	E_{IN}* *liters*	ΔE_{IN} *liters*	Na *mEq./L.*	Cl *mEq./L.*	K *mEq./L.*	Na *mEq.*	ΔNa *mEq.*	Cl *mEq.*	ΔCl *mEq.*	K *mEq.*	ΔK *mEq.*	Na *mEq.*	Cl *mEq.*	K' *mEq.*	$\Delta Na_{IN} - b_{Na}$ *mEq.*	$\Delta Cl_{IN} - b_{Cl}$ *mEq.*	$\Delta K_{IN} - b_{K'}$ *mEq.*
J. V.	2	12.5	0	183	48 hr.	12.5/d p.o.	6/d	183	+ .2	10.5 12.9	+2.4	138.4 137.5	105.9 101.6	6.12 5.91	1453 1774	+321	1112 1369	+257	64 76	+12	+100	+ 19	+25	221	238	−13
T. S.	2	0	6	113	24 hr.	75/d p.o.	0	216	+ .5	12.6 13.4	+0.8	141.1 148.8	117.6 120.8	5.33 5.76	1778 1994	+216	482 1619	+135	67 77	+10	+127	+106	+11	89	2	− 1
M. H.	2	0	0	108	16 hr.	150‡ i.v.	0	323	+1.4	6.9 9.6	+2.7	134.7 136.3	99.6 101.4	5.92 5.90	929 1308	+379	687 973	+286	41 57	+16	+120	+162	−37	259	124	53
B. G.	2	0	0	118	46 min.	10§ i.v.	0	X		7.9 9.7	+1.8	142.0 143.8	110.8 114.0	X X	1122 1395	+273	876 1106	+230		X	X	X	X	273	230	X
M. R.	2	0	1	190	67 min.	9§ i.v.	0	X		5.5 5.4	−0.1	143.4 143.4	109.5 109.5	X X	788 774	− 14	602 591	− 11		X	X	X	X	−14	−11	X
J. C.	2	0	0	180	50 min.	24§ i.v.	0	X		4.8 5.9	+1.1	142.4 141.4	115.9 115.0	X X	683 834	+151	556 678	+122		X	X	X	X	151	122	X
S. C.	X	0	0	X	58 min.	16§ i.v.	0	X		10.3 10.2	−0.1	137.0 140.0	117.0 114.9	X X	1411 1397	− 14	1205 1173	− 32		X	X	X	X	−14	−32	X

* Experimental values appear below their controls.
† Minus values represent transfers out of inulin space.
‡ Hydrocortisone free alcohol in 5 per cent dextrose, kindly supplied by Dr. Elmer Alpert of Merck and Co.
§ Cortisone free alcohol in physiologic saline, also supplied by Dr. Elmer Alpert of Merck and Co.

TABLE VIII

Calculated transfers of water and electrolyte out of the inulin space but not out of the chloride space in acute adrenal insufficiency

Patient	$\Delta E_{Cl} - \Delta E_{IN}$ *liters*	$\Delta Na_{Cl} - \Delta Na_{IN}$ *mEq.*	$b_{Cl} - \Delta Cl_{IN}$ *mEq.*	$\Delta K_{Cl} - \Delta K_{IN}$ *mEq.*
H. L.	1.4	175	144	8
B. G.	1.2	199	154	7
M. R.	1.1	158	128	6
J. C.	2.0	285	245	9
S. W.	4.5	626	525	26
T. S.	1.8	260	205	10
M. H.	1.2	162	116	7
Mean	1.89	266.4	216.7	10.4
S.E.M.	.45	62.7	54.2	2.6
t	4.20	4.25	4.00	4.00
P	<.01	<.01	<.01	<.01

It would, therefore, appear that hyponatremia, when it occurs in acute adrenal insufficiency, is ascribable primarily to renal wastage of sodium. The effectiveness of net external loss of sodium in reducing the concentration of sodium in extracellular fluid would, however, be enhanced by any concurrent depletion of the volume and total sodium content of extracellular fluid brought about by internal transfers of fluid.

The plan of the experiments in which steroid was withdrawn from adrenal-deficient patients was designed to disclose information concerning *acute* adrenal insufficiency.[7] Our reported observations may well not be applicable to subacute or chronic adrenal insufficiency resulting from a more prolonged and less severe lack of hormone in relation to requirement; and indeed weight loss is generally regarded as a cardinal and even indispensible feature of the latter conditions.

Withdrawal of replacement steroid from the adrenal-deficient subject is a valid method for the experimental study of adrenal insufficiency, and indeed the only feasible way to study uncomplicated acute adrenal insufficiency. Since the patients were receiving during the control period approximately the minimum exogenous hormone capable of maintaining their well-being, it is proper to emphasize that the fluid transfers observed are not to be regarded as effects of withdrawal of steroids exhibited in pharmacologic quantity. The transfers were observed whether or not dietary salt was reduced when hormone was withdrawn (Table I). Similar transfers did not accompany drastic dietary salt reduction in the hypertensive subject with intact adrenal glands; nor have they occurred, under the standard conditions employed in the present series, in subtotally adrenalectomized subjects manifesting evidence of adrenocortical reserve (Table VI). They are therefore, so far as can be determined by the available data, a specific effect of acute adrenal insufficiency. They are moreover preventable or reversible indiscriminately by desoxycorticosterone, hydrocortisone, or cortisone; and the latter compound, at least, can apparently moderate or even rectify the abnormal fluid distribution of acute adrenal insufficiency within as short a time as an hour (Table VII).

There is little reason to suppose that the coexistence of vascular disease modified the effects of induced acute adrenal insufficiency upon fluid distribution in these patients, though of course this possibility may be kept in mind. Certainly the changes observed are quite similar to those reported in normal dogs (5, 6). Gaudino and Levitt (5) indeed reported much larger percentile decreases of the inulin space in dogs developing acute adrenal insufficiency; the difference may well be ascribable to a difference in the severity of acute adrenal insufficiency which was permitted to develop in canine and human subjects, respectively.

More precise characterization of the fluid and electrolyte transfers which occurred in these patients depends upon the anatomical boundaries which are to be assigned to the volumes of distribution of inulin and of chloride. Although the volumes of distribution of these substances have both been extensively used as approximations of extracellular fluid volume, objections have been urged against the use of each method for this purpose, and definition of the anatomical boundaries corresponding to these volumes is a matter of controversy.

Chloride is admittedly present in the cells of certain tissues (29, 30); the justification for the use of its volume of distribution as a measure of extracellular fluid volume must therefore rest upon the premise that intracellular chloride is either so small in quantity as compared with extracellular

[7] There is need in endocrinology for two terms which will be generally acceptable as expressing two distinct concepts: 1) lack of functioning endocrine tissue, and 2) physiologic consequences resulting from such lack. In this communication, for want of better terminology, we understand by adrenal insufficiency, manifestations attributable to lack of adrenal hormone in relation to need, and by adrenal deficiency, simple deficit of adrenal tissue.

chloride, or that the quantity of exchangeable intracellular chloride changes so little under the conditions of any particular study, or both, that the error in estimating extracellular fluid changes as the change of the chloride space is small. Hastings (30) reviewed the extensive though necessarily indirect evidence which led to rather general acceptance of such assumptions. Conclusive direct evidence as to the quantity, and of the stability of cell chloride under pathological circumstances, is nevertheless lacking. It has been maintained (31) that a very large proportion of total body chloride—as much as a third—is intracellular; but the chief support for this contention is derived from unqualified acceptance of the identity of extracellular fluid volume and the volume of distribution of inulin—an assumption which is itself open to question.

When inulin is administered to man by the technique employed in these experiments, equilibrium distribution in a virtually constant limiting fluid volume is said to be attained (in the absence of abnormal fluid collections) at the end of a five-hour infusion. This finding, together with evidence that inulin does not pass cell membranes, affords the chief basis for the view of some investigators (15, 32, 33, 34) that the inulin space corresponds to extracellular fluid volume. It is not clear, however, that any satisfactory evidence has been cited to exclude the possibility that the volume of distribution of inulin may correspond to a subcompartment of the extracellular fluid. Nichols, Nichols, Weil, and Wallace (35) concluded, on the basis of analytical studies of particular tissues following inulin administration to dogs, that the inulin space is a less satisfactory measure of extracellular fluid volume than the chloride space, owing to deficient penetration of inulin into connective tissue. One may accordingly entertain the alternative view that the chloride space comprises the entire inulin space and in addition includes a connective tissue compartment exhibiting essentially the chemical characteristics of extracellular fluid modified by the presence of connective tissue proteins.

If the inulin space is accepted as a valid measurement of extracellular fluid volume,[8] the data would indicate intracellular migration of large quantities of water, sodium, chloride, and potassium in acute adrenal insufficiency, the quantities transferred into cells being given by deducting from the total decrease of the inulin space and its contained ions the net external loss of these substances ($\Delta W - \Delta E_{IN}$, Table V; $b_{Na} - \Delta Na_{IN}$ and $b_{Cl} - \Delta Cl_{IN}$, Table III) or adding the net external gain ($b_{K'} - \Delta K_{IN}$, Table III). A significant excess of sodium over chloride is transferred (transferred Na − Cl, Table III). These ions move in approximately the proportion of their relative concentrations in extracellular fluid; while potassium and water, according to their proportions in extracellular fluid relative to sodium or chloride, are transferred in excess. Transfers in the opposite sense would be effected by adrenal steroids (Table VII).

If, on the other hand, the chloride space affords a valid approximation to extracellular fluid volume, and if it exceeds the inulin space by virtue of including a large volume of connective tissue water, the interpretation of the data is somewhat more complex. The decrease of the chloride space, and of the calculated sodium and potassium of the chloride space, after correction for external balance, will represent transfers into the cellular compartment ($\Delta W - \Delta E_{Cl}$, Table V; $b_{Na} - \Delta Na_{Cl}$, $b_{K'} - \Delta K_{Cl}$, Table IV). The decrease of the inulin space in excess of the decrease of the chloride space will represent transfer of fluid out of the inulin space, but not out of the chloride space ($\Delta E_{Cl} - \Delta E_{IN}$, Table VIII)[9];

[8] On this assumption, it is not possible for any effects of a disease state on cell permeability to produce a factitial *decrease* of the inulin space, since, according to hypothesis, there is normally no penetration beyond the extracellular phase throughout which inulin is homogeneously diffused. Any abnormal penetration of inulin into cells would cause a factitial *increase* of the apparent inulin space.

[9] An alternate interpretation of the discrepancy between the magnitudes of change of the inulin space and the chloride space would ascribe to adrenal hormones an effect upon the penetrability to inulin of the connective tissue. On this view, it would be supposed that in adrenal insufficiency inulin gains access to connective tissue water to a much lesser extent than in the normal individual, so that the apparent decrease of the inulin space would be artifactual. There is no way to exclude this possibility; but it must be said that one is still confronted with the necessity of attributing to adrenal hormones profound effects upon the physicochemical state of collagen, and that the internal transfers of fluid as calculated on the basis of the chloride space are not brought into question by this hypothesis.

and the calculated electrolyte losses from the inulin space in excess of electrolyte losses from the chloride space will similarly represent transfer of these ions out of the inulin space, but not out of the chloride space ($\Delta Na_{Cl} - Na_{IN}$, $b_{Cl} - \Delta Cl_{IN}$, $\Delta K_{Cl} - \Delta K_{IN}$, Table VIII). According to the hypothesis, the figures will express the magnitudes of transfer of water and electrolytes from the inulin space into connective tissue.

If this interpretation of the data is adopted, the figures imply that in every instance the bulk of the fluid transfer in acute adrenal insufficiency is attributable to increased hydration of the connective tissue brought about by movement of water, sodium, chloride, and potassium in quantities closely corresponding to their relative proportions in extracellular fluid. Movement into the cellular compartment of water and of large amounts of potassium, without transfer of sodium, would also be implied.

This second interpretation of the data agrees with reports based on tissue analysis, in that the sodium apparently transferred from extracellular fluid in adrenal insufficiency could never be found in the cells of any of the soft tissues examined (skeletal muscle, heart muscle, liver and kidney), and would suggest the connective tissue as the site of the missing sodium. The agreement was perhaps to be expected, inasmuch as the chloride space was used as the measure of extracellular fluid volume when calculations of cell composition were applied to the reported tissue studies. Direct chemical analysis of the connective tissue of animals dying in acute adrenal insufficiency would be of great interest. If a greatly increased content of water and sodium of this tissue were found to accompany adrenal insufficiency, support would be provided for the utility of simultaneous estimates of the changes in inulin and chloride space in studying fluid distribution in the intact organism, and for the view of Nichols *et al.* that the inulin space does not include some of the plasma-ultrafiltrate phase of connective tissue water. Ludwig, Chen, and Soffer (36) have observed that the hydration of connective tissue is affected by certain steroid hormones; and adrenal cortical regulation of the hydration of connective tissue would be a matter of considerable interest in view of the spectacular effects of cortisone upon diseases affecting collagenous tissue.

SUMMARY

1. Acute adrenal insufficiency induced in seven adrenal-deficient patients was attended by a significant decrease of the chloride space (calculated) and a much larger decrease of the inulin space (measured).

2. The calculated net loss of potassium and water from the chloride space, and of sodium, chloride, potassium, and water from the inulin space, could not be attributed to net external loss. Internal transfers of fluid and electrolytes, therefore, accompany acute adrenal insufficiency in man.

3. Interpretation of the nature of the observed transfers depends upon the anatomical boundaries to be assigned the volumes of distribution of chloride and of inulin. Alternative interpretations of the data are presented.

4. Desoxycorticosterone, hydrocortisone, and cortisone are all individually capable of preventing or correcting the abnormal distribution of body fluid characteristic of acute adrenal insufficiency; in the case of intravenous cortisone, corrective effects may be observed within an hour.

ACKNOWLEDGMENTS

We wish to express our thanks to Drs. J. Russell Elkinton and William A. Jeffers for their helpful advice and support.

We are indebted, also, to Miss Helen Merton, Mrs. Cynthia Henderson, and the nursing staff of the Metabolic Unit, and to Miss Marie Wackermann, who was wholly responsible for the dietary management. Miss Catherine Macatsoris, Mr. William Luetzell, Mrs. Kathryn Boyer, and Miss Virginia Alexa contributed technical assistance.

Dr. William Fitts performed the adrenalectomy in S. W.; all the other adrenal resections were done by Dr. Harold A. Zintel.

Maintenance of hormone therapy for our postadrenalectomy patients has been supplied *gratis* through the kindness of Drs. Elmer Alpert and Augustus Gibson of Merck and Company (cortone®); Dr. F. L. Mohr, of Ciba Pharmaceutical Products, Inc. (percorten®); and Dr. R. W. Burlew, of Schering Corporation (cortate®).

REFERENCES

1. Swingle, W. W., Pfiffner, J. J., Vars, H. M., and Parkins, W. M., The effect of fluid deprivation and fluid intake upon the revival of dogs from adrenal insufficiency. Am. J. Physiol., 1934, **108**, 144.
2. Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W., Relation of serum sodium and chloride levels to alterations of body water in the intact and adrenalectomized dog, and the influence

of adrenal cortical hormone upon fluid distribution. Am. J. Physiol., 1936, **116**, 438.

3. Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W., A study of water intoxication in the intact and adrenalectomized dog and the influence of adrenal cortical hormone upon fluid and electrolyte distribution. Am. J. Physiol., 1937, **119**, 557.
4. Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W., The influence of adrenal cortical hormone upon electrolyte and fluid distribution in adrenalectomized dogs maintained on a sodium and chloride free diet. Am. J. Physiol., 1937, **119**, 684.
5. Gaudino, M., and Levitt, M. F., Influence of the adrenal cortex on body water distribution and renal function. J. Clin. Invest., 1949, **28**, 1487.
6. Flanagan, J. B., Davis, A. K., and Overman, R. R., Mechanism of extracellular sodium and chloride depletion in the adrenalectomized dog. Am. J. Physiol., 1950, **160**, 89.
7. Harrison, H. E., and Darrow, D. C., The distribution of body water and electrolytes in adrenal insufficiency. J. Clin. Invest., 1938, **17**, 77.
8. Darrow, D. C., Harrison, H. E., and Taffel, M., Tissue electrolytes in adrenal insufficiency. J. Biol. Chem., 1939, **130**, 487.
9. Muntwyler, E., Mellors, R. C., Mautz, F. R., and Mangun, G. H., Electrolyte and water equilibria in the dog. II. Electrolyte and water exchange between skeletal muscle and blood in adrenal insufficiency. J. Biol. Chem., 1940, **134**, 367.
10. Buell, M. V., and Turner, E., Cation distribution in the muscles of adrenalectomized rats. Am. J. Physiol., 1941, **134**, 225.
11. Swingle, W. W., and Remington, J. W., The role of the adrenal cortex in physiological processes. Physiol. Rev., 1944, **24**, 89.
12. Stern, T. N., Cole, V. V., Bass, A. C., and Overman, R. R., Dynamic aspects of sodium metabolism in experimental adrenal insufficiency using radioactive sodium. Am. J. Physiol., 1951, **164**, 437.
13. Overman, R. R., Sodium, potassium and chloride alterations in disease. Physiol. Rev., 1951, **31**, 285.
14. Hills, A. G., Webster, G. D., Rosenthal, O., Dohan, C. F., Richardson, E. M., Zintel, H. A., and Jeffers, W. A., Quantitative evaluation of primary adrenal cortical deficiency in man. Am. J. Med., (In press).
15. Schwartz, I. L., Schachter, D., and Freinkel, N., The measurement of extracellular fluid in man by means of a constant infusion technic. J. Clin. Invest., 1949, **28**, 1117.
16. Eisenman, A. J., Mackenzie, L. B., and Peters, J. P., Protein and water of serum and cells of human blood, with a note on the measurement of red blood cell volume. J. Biol. Chem., 1936, **116**, 33.
17. Elkinton, J. R., and Winkler, A. W., Transfers of intracellular potassium in experimental dehydration. J. Clin. Invest., 1944, **23**, 93.
18. Lavietes, P. H., The metabolic measurement of the water exchange. J. Clin. Invest., 1935, **14**, 57.
19. Long, J. H., On the relation of the specific gravity of urine to the solids present. J. Am. Chem. Soc., 1903, **25**, 257.
20. Snedecor, G. W., Statistical Methods Applied to Experiments in Agriculture and Biology, ed. 4, Ames, Iowa State College Press, 1946.
21. Harrison, H. E., A modification of the diphenylamine method for determination of inulin. Proc. Soc. Exper. Biol. & Med., 1942, **49**, 111.
22. Wallace, W. M., Holliday, M., Cushman, M., and Elkinton, J. R., The application of the internal standard flame photometer to the analysis of biologic material. J. Lab. & Clin. Med., 1951, **37**, 621.
23. Eisenman, A. J., A note on the Van Slyke method for the determination of chlorides in blood and tissue. J. Biol. Chem., 1929, **82**, 411.
24. Bonsnes, R. W., and Taussky, H. H., On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 1945, **158**, 581.
25. Karr, W. G., A method for the determination of blood urea nitrogen. J. Lab. & Clin. Med., 1924, **9**, 329.
26. Wolfson, W. Q., Cohn, C., Calvary, E., and Ichiba, F., Studies in serum proteins. A rapid procedure for the estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin, and gamma globulin in 1.0 ml. of serum. Am. J. Clin. Path., 1948, **18**, 723.
27. Talbott, J. H., Pecora, L. J., Melville, R. S., and Consolazio, W. V., Renal function in patients with Addison's disease and in patients with adrenal insufficiency secondary to pituitary pan-hypofunction. J. Clin. Invest., 1942, **21**, 107.
28. Waterhouse, C., and Keutmann, E. H., Kidney function in adrenal insufficiency. J. Clin. Invest., 1948, **27**, 372.
29. Manery, J. F., and Hastings, A. B., The distribution of electrolytes in mammalian tissues. J. Biol. Chem., 1939, **127**, 657.
30. Hastings, A. B., The electrolytes of tissues and body fluids. Harvey Lect., 1940–41, **36**, 91.
31. Steele, J. M., Body water. Am. J. Med., 1950, **9**, 141.
32. Gaudino, M., Schwartz, I. L., and Levitt, M. F., Inulin volume of distribution as a measure of extracellular fluid in dog and man. Proc. Soc. Exper. Biol. & Med., 1948, **68**, 507.
33. Gaudino, M., and Levitt, M. F., Inulin space as a measure of extracellular fluid. Am. J. Physiol., 1949, **157**, 387.
34. Levitt, M. F., and Gaudino, M., Measurement of body water compartments. Am. J. Med., 1950, **9**, 208.
35. Nichols, G., Jr., Nichols, N., Weil, W. B., and Wallace, W. M., The direct measurement of the extracellular phase of tissues. J. Clin. Invest., 1952, **31**, 652.
36. Ludwig, A. W., Chen, D. K., and Soffer, L. J., The relationship between connective tissue mucopolysaccharide content and tissue electrolyte composition. J. Clin. Endocrinol. & Metab., 1952, **112**, 965.

ERYTHROCYTE METABOLISM. THE METABOLIC BEHAVIOR OF THE CHOLESTEROL OF HUMAN ERYTHROCYTES [1]

By IRVING M. LONDON [2] AND HAIYIM SCHWARZ

(From the Department of Medicine, Columbia University, College of Physicians and Surgeons and the Presbyterian Hospital, New York, N. Y.)

(Submitted for publication July 21, 1953; accepted August 11, 1953)

In the course of studies on the ageing process in the human erythrocyte, we have investigated the metabolic behavior of the cholesterol of the erythrocyte. This study was designed to determine whether the cholesterol of the human erythrocyte is in the dynamic state (2), or whether the cholesterol is incorporated into the newly formed erythrocyte and remains with the cell throughout the cell's life span, as does hemoglobin (3–6).

A previous study (7) has shown that the cholesterol of the plasma is in the dynamic state. The rate of entry of cholesterol into the plasma of the circulating blood was measured by determining the rate at which deuterium is incorporated into the cholesterol of the plasma when an elevated concentration of deuterium in the body water is maintained at a steady level by repeated administration of deuterium oxide. In the studies described in this report, the same technique has been employed and the concentrations of deuterium in the cholesterol of the erythrocytes and of the plasma have been compared. Since the cholesterol of the plasma is in the dynamic state, the finding of a similar rate of turnover and a similar pattern of concentrations of deuterium in the cholesterol of the erythrocytes would favor the conclusion that the cholesterol of the erythrocytes is also in the dynamic state.

The subjects of this study were a patient with the nephrotic syndrome and a patient with xanthomatosis tuberosum multiplex. A discussion of the mechanisms involved in the maintenance of hypercholesterolemia in these conditions is beyond the scope of this paper and will not be presented here.

MATERIAL AND METHODS

Subjects: 1) F. G., a 36 year old Negro woman, was studied during the nephrotic phase of chronic glomerulonephritis. Pertinent laboratory data included a red blood cell count of 3.5 million per cubic millimeter, hemoglobin of 10.0 Gm. per 100 ml. of blood, and venous hematocrit of 33 per cent; plasma total cholesterol 836 mg. per cent with 70 per cent of the total cholesterol esterified; erythrocyte total cholesterol 146 mg. per cent with 11 per cent of the total in the ester form and 89 per cent in the form of free cholesterol.

2) M. T., a 21 year old white woman with xanthoma tuberosum multiplex, was essentially asymptomatic. Hematologic data included a red blood cell count of 4.2 million per cubic millimeter and a hemoglobin value of 13.0 Gm. per 100 ml. of blood. Plasma total cholesterol was found to range between 500 mg. per cent and 660 mg. per cent with 71 to 75 per cent of the total esterified. Erythrocyte total cholesterol was 132 mg. per cent with 95 per cent of the total in the form of free cholesterol and 5 per cent in ester form.

Administration of deuterium oxide: 1) Subject F. G. drank 1000 ml. of 50 per cent D_2O in four divided doses on the first day of the experiment and then received 60 to 65 ml. per day of approximately 30 per cent D_2O for the next 112 days. On 11 occasions the daily dose was doubled in order to maintain the body water concentration of deuterium at a steady level. The concentration of deuterium in the body water was measured by determining the concentration of deuterium in the water of the urine.

2) Subject M. T. drank 666 ml. of 50 per cent D_2O in three divided doses on the first day of the experiment and 50 to 60 ml. of 50 per cent D_2O daily for the duration of the study.

Isolation and quantitative determination of cholesterol: The erythrocytes were washed three times with 0.9 per cent solution of sodium chloride. The buffy coat of leucocytes was removed and discarded. Total cholesterol was isolated as the digitonide from the unsaponifiable fractions of erythrocytes and of plasma and was then recovered from the digitonide according to the method of Schönheimer and Dam (8). Melting point determinations were used as an index of purity.

Quantitative determinations of cholesterol were per-

[1] This work was presented in part before the American Society for Clinical Investigation at Atlantic City, May 1951 (1). The work was supported by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council and by a grant from the Office of Naval Research.

[2] Part of this work was performed during the tenure of a Welch Fellowship in Internal Medicine of the National Research Council.

formed according to the Sperry-Schoenheimer method (9, 10).[3]

Measurement of concentration of deuterium: The concentration of deuterium in the hydrogen derived from the cholesterol was determined according to the procedure of Graff and Rittenberg (11).[4]

Synthesis of cholesterol in vitro: Two hundred and seventy ml. of blood were drawn from a normal man, the plasma and buffy coat were removed by centrifugation, the erythrocytes were suspended in 0.9 per cent sodium chloride solution, the suspension was centrifuged once, and the saline and the remaining buffy coat were discarded. The erythrocytes were suspended in saline to make a total volume of 280 ml. Two hundred mg. of 1-C^{14}-sodium acetate, $CH_3C^{14}OONa \cdot 3H_2O$, with a specific activity of 0.8 millicuries per millimole, was added to the cell-saline suspension. Penicillin and streptomycin were added to inhibit bacterial growth. The suspension was incubated for 25 hours with constant gentle shaking in air at 38°C.

RESULTS AND DISCUSSION

The concentrations of deuterium in the cholesterol of the plasma and of the erythrocytes of both subjects are plotted in Figures 1 and 2. In each case, the rise in the concentration of deuterium in the cholesterol of the erythrocytes is similar to its rise in the cholesterol of the plasma. Since the cholesterol of the plasma is in the dynamic state, a similar rate of turnover in the cholesterol of the erythrocytes favors the conclusion that the cholesterol of the erythrocytes is also in the dynamic state. There are two possible mechanisms whereby the cholesterol of the erythrocyte could exist in a dynamic state: the first would require the synthesis of cholesterol by the circulating erythrocyte for replacement of cholesterol which has disappeared from the erythrocyte; the second would entail the replacement of the cholesterol of the erythrocyte by cholesterol from the plasma.

The first possibility, synthesis by the erythrocyte, was investigated by determining the capacity of mature human erythrocytes to synthesize cholesterol *in vitro,* as measured by the C^{14} concentration in the cholesterol of erythrocytes incubated with 1-C^{14}-sodium acetate ($CH_3C^{14}OONa$). The results of this experiment are presented in Table I.

That this synthesis of cholesterol by erythrocytes *in vitro* is relatively insignificant can better be appreciated by comparison of these results with the *in vitro* synthesis of cholesterol in rat liver. On incubation of 60 Gm. of rat liver slices with 0.15 millicuries (25 mg.) of $CH_3C^{14}OONa \cdot 3H_2O$ for 3.5 hours, 7 per cent of the C^{14} labeled sodium acetate was converted to cholesterol. We recognize that it may be hazardous to conclude, on the basis of *in vitro* studies, that the mature human erythrocyte *in vivo* possesses only a very limited capacity to synthesize cholesterol. The contrast, however, between the active turnover of cholesterol of the erythrocyte and the very low rate of synthesis of cholesterol by the erythrocyte *in vitro* is sufficiently marked to favor the view that the existence of the cholesterol of the erythrocyte in the dynamic state is due to a process other than synthesis of cholesterol by the erythrocyte itself. The conclusion which is indicated by these findings is that the cholesterol of the erythrocyte is in dynamic exchange with the cholesterol of the plasma.

This conclusion is in agreement with studies performed simultaneously and independently in other laboratories. Determinations of C^{14} activity in the cholesterol of a rabbit's erythrocytes after the administration of $CH_3C^{14}OONa$ indicated that the cholesterol of the erythrocytes is in the dynamic state (12). Studies *in vitro* of dog erythrocytes and plasma have shown a rapid equilibration of the cholesterol of the erythrocytes with the free cholesterol of the plasma (13, 14). The administration of tritium (H^3) labeled cholesterol (15) or of C^{14} labeled cholesterol (16) to man has revealed equilibration of cholesterol of the erythrocytes with the free cholesterol of the plasma.

Inasmuch as the major part of the cholesterol of erythrocytes is not esterified, equilibration of cholesterol of erythrocytes with the cholesterol of the plasma might be expected to involve the free cholesterol principally. It would seem likely, however, that the esterified cholesterol of the erythrocyte, which is present in low concentration, would also be in equilibrium with the esterified cholesterol of the plasma. The technique of D_2O administration which was employed in this study would not be expected to reveal different isotope concentrations in free and esterified cholesterol of plasma. Consequently, the isotope concentrations in total cholesterol of erythrocytes and of plasma respectively were compared.

[3] We are indebted for these determinations to Mr. M. L. Webb, in the laboratory of Dr. Warren M. Sperry, and to Miss Anne Richards, in the laboratory of Dr. Kenneth B. Turner.

[4] Analyses in the mass spectrometer were kindly performed by Mr. Irving Sucher.

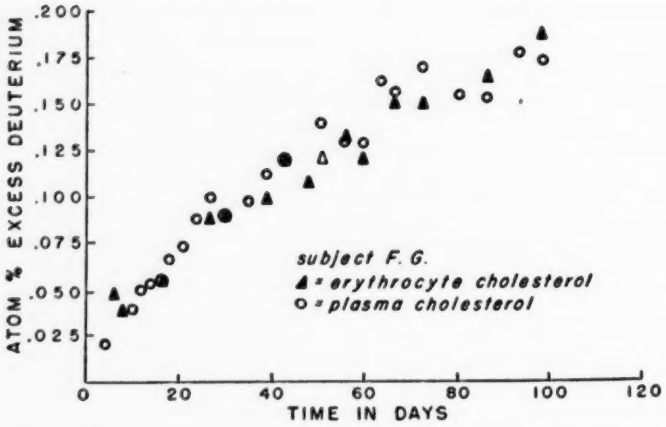


FIG. 1. CONCENTRATIONS OF DEUTERIUM IN THE TOTAL CHOLESTEROL OF ERYTHROCYTES AND OF PLASMA

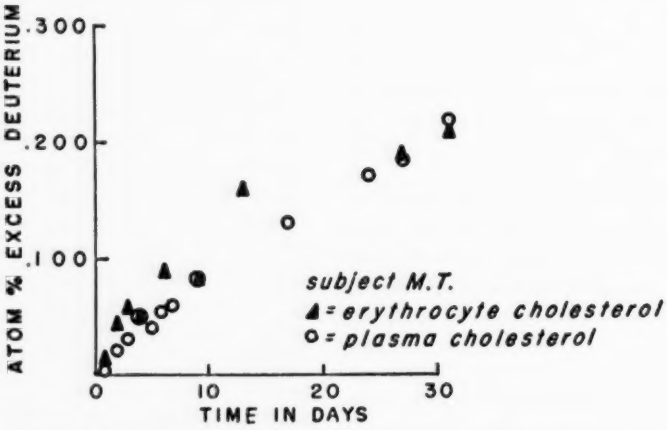


FIG. 2. CONCENTRATIONS OF DEUTERIUM IN THE TOTAL CHOLESTEROL OF ERYTHROCYTES AND OF PLASMA

The demonstration that the cholesterol of the mature human erythrocyte is renewed by exchange with cholesterol of the plasma is pertinent to the problem of ageing of the human erythrocyte. Several different techniques have established that the normal human erythrocyte survives for an average life span of about 120 days (3, 5, 17, 18). In the course of some of these and related studies (3–6, 18), it has been shown that the hemoglobin of the mature circulating erythrocyte is not in the dynamic state, but rather that it remains with the erythrocyte until the cell is destroyed. The determination of the metabolic behavior of hemoglobin and of other constituents of the human erythrocyte in normal and in pathologic states may provide some clues to an understanding of the ageing process in this cell. The behavior of constituents of the stroma of the erythrocyte may be particularly significant in determining the viability of the cell.

The stroma is composed, at least in part, of lipoprotein complexes. The cholesterol of the erythrocyte is, most likely, bound to one or more proteins of the stroma and is probably part of one or more lipoprotein complexes. The evidence presented in this and other studies indicates that the cholesterol is metabolically labile. Earlier studies

TABLE I

Synthesis of cholesterol in vitro by mature human erythrocytes

Weight (wet) of erythrocytes incubated	Amount of $CH_4C^{13}OONa \cdot 3H_2O$ added	Total C^{14} activity added	Time of incubation	C^{14} activity* of cholesterol	Per cent of C^{14} acetate converted to cholesterol
Gm.	*mg.*	*millicuries*	*hours*	*c.p.m.*	
120	200	1.2	25	21	0.0004

* The C^{14} activity is expressed as counts per minute above background for an infinitely thick sample.

(19, 20) and findings in this laboratory on the metabolism of phospholipids in the erythrocytes of man (21) indicate a rapid turnover of total phospholipids, as well, in these cells. The metabolic behavior of the stroma proteins is not yet known but a preliminary report suggests that most of the proteins in the stroma of the rat erythrocytes are, once formed, as stable metabolically as hemoglobin (22).

It is tempting to speculate on the relevance of these observations to the mechanisms which are responsible for maintaining the viability of the cell, and, more particularly, for maintaining the integrity of the erythrocyte membrane. The maintenance of an intact membrane may require a continuing rapid exchange of cholesterol and other lipids with similar constituents in the plasma, and, at the same time, may require metabolic stability of some proteins of the stroma. It is possible that one or more proteins of the stroma are in the dynamic state, and the integrity of the cell structure may be dependent on a continued replacement of these proteins. In any case, the structure of the human erythrocyte appears to depend on the maintenance of some constituents in a dynamic state and of others in an unreactive or stable state. The death of the erythrocyte may result from loss of metabolic lability of some constituents or from loss of metabolic stability of others. Further definition of the metabolic behavior of various constituents in erythrocytes of different ages may provide illuminating information on the process of ageing in the human erythrocyte.

SUMMARY

In two human subjects, in whose body water an elevated concentration of deuterium was maintained by the administration of deuterium oxide, the rise in the concentration of deuterium in the cholesterol of the erythrocytes was similar to its rise in the cholesterol of the plasma. This finding and the demonstration of a relatively insignificant degree of synthesis of cholesterol by human erythrocytes *in vitro* favor the conclusion that the cholesterol of human erythrocytes is in dynamic exchange with the cholesterol of the plasma. The possible relevance of the metabolic lability of the cholesterol of erythrocytes to the problem of the ageing process in the human erythrocyte is indicated.

REFERENCES

1. London, I. M., Sabella, G. F., and Yamasaki, M. M., Studies on the metabolism of cholesterol in normal man and in the nephrotic syndrome. J. Clin. Invest., 1951, **30**, 657.
2. Schoenheimer, R., The dynamic state of body constituents, ed. 2, Cambridge, Harvard University Press, 1946.
3. Shemin, D., and Rittenberg, D., The life span of the human red blood cell. J. Biol. Chem., 1946, **166**, 627.
4. London, I. M., Shemin, D., West, R., and Rittenberg, D., Heme synthesis and red blood cell dynamics in normal humans and in subjects with polycythemia vera, sickle-cell anemia, and pernicious anemia. J. Biol. Chem., 1949, **179**, 463.
5. Jope, E. M., The disappearance of sulphemoglobin from the blood of TNT workers in relation to the dynamics of red cell destruction. Brit. J. Indust. Med., 1946, **3**, 136.
6. Grinstein, M., Kamen, M. D., and Moore, C. V., The utilization of glycine in the biosynthesis of hemoglobin. J. Biol. Chem., 1949, **179**, 359.
7. London, I. M., and Rittenberg, D., Deuterium studies in normal man. I. The rate of synthesis of serum cholesterol. II. The measurement of total body water and water absorption. J. Biol. Chem., 1950, **184**, 687.
8. Schönheimer, R., und Dam, H., Über die spaltbarkeit und löslichkeit von sterindigitoniden. Ztschr. f. physiol. Chem., 1933, **215**, 59.
9. Schoenheimer, R., and Sperry, W. M., A micromethod for the determination of free and combined cholesterol. J. Biol. Chem., 1934, **106**, 745.

10. Sperry, W. M., and Webb, M., A revision of the Schoenheimer-Sperry method for cholesterol determination. J. Biol. Chem., 1950, **187**, 97.
11. Graff, J., and Rittenberg, D., Microdetermination of deuterium in organic compounds. Analytical Chemistry, 1952, **24**, 878.
12. Muir, H. M., Perrone, J. C., and Popják, G., Studies on the metabolism of the circulating erythrocyte in the rabbit. Biochem. J., 1951, **48**, iv.
13. Gould, R. G., Lipid metabolism and atherosclerosis. Am. J. Med., 1951, **11**, 209.
14. Hagerman, J. S., and Gould, R. G., The 'in vitro' interchange of cholesterol between plasma and red cells. Proc. Soc. Exper. Biol. & Med., 1951, **78**, 329.
15. Biggs, M. W., Kritchevsky, D., Colman, D., Gofman, J. W., Jones, H. B., Lindgren, F. T., Hyde, G., Lyon, G., and Lyon, T. P., Observations on the fate of ingested cholesterol in man. Circulation, 1952, **6**, 359.
16. Hellman, L., Rosenfeld, R., Fukushima, D., Eidinoff, M., Brunschwig, A., and Gallagher, T., The major pathways of steroid metabolism in man. J. Clin. Invest., 1953, **32**, 573.
17. Ashby, W., The span of life of the red blood cell. A résumé. Blood, 1948, **3**, 486.
18. Finch, C. A., Wolff, J. A., Rath, C. E., and Fluharty, R. G., Iron metabolism. Erythrocyte iron turnover. J. Lab. & Clin. Med., 1949, **34**, 1480.
19. Hahn, L., and Hevesy, G., Phosphatide exchange between plasma and organs. Nature, 1939, **144**, 204.
20. Zilversmit, D. B., Entenman, C., Fishler, M. C., and Chaikoff, I. L., The turnover rate of phospholipids in the plasma of the dog as measured with radioactive phosphorus. J. Gen. Physiol., 1943, **26**, 333.
21. Unpublished data from this laboratory.
22. Muir, H. M., Neuberger, A., and Perrone, J. C., Further isotopic studies on haemoglobin formation in the rat and rabbit. Biochem. J., 1952, **52**, 87.

STUDIES ON THE COMPOSITION OF THE FIBRINOID MATERIAL OF THE SUBCUTANEOUS NODULE OF RHEUMATOID ARTHRITIS [1]

By MORRIS ZIFF, THOMAS KANTOR, EDWARD BIEN,[2] AND ALFRED SMITH

(From the Study Group on Rheumatic Diseases and the Departments of Medicine and Pathology, New York University College of Medicine, New York, N. Y.)

(Submitted for publication June 19, 1953; accepted August 18, 1953)

Fibrinoid has been described as a "homogeneous, eosinophilic, refractile, relatively acellular, band-like structure which has some of the tinctorial properties of fibrin" (1, 2). It is found in the connective tissue in a number of diseases, especially in the group of "diffuse collagen diseases" (3–5), including rheumatic fever and rheumatoid arthritis (6, 7), lupus erythematosus and scleroderma (3, 8–10), and periarteritis nodosa (11). In these diseases, the presence of fibrinoid material in close association with the fibrillar structures of the connective tissue is perhaps, histologically, the single common denominator (4, 5). Altschuler and Angevine (1, 2) have listed numerous other sites of occurrence of fibrinoid which include the base of peptic ulcers (12), the normal placenta (13), and lesions associated with hypergic inflammation (14). The production of fibrinoid by a variety of experimental stimuli has been described; these include pinching of tissue (15), orthostatic hypotension (16), prolonged passive hyperemia (17), and massive administration of desoxycorticosterone (18).

Our present knowledge about fibrinoid is derived almost exclusively from studies of its histological staining characteristics. The latter are in most instances similar to those of fibrin, hence the name fibrinoid; and it has indeed been suggested (16, 19) that fibrinoid consists of inspissated fibrin and other blood proteins. Since fibrinoid often seems to blend with ragged or split bundles of otherwise normal-appearing collagen, Bahrmann (20) concluded that fibrinoid resulted from infiltration of degenerated collagen fibers by fibrin.

Klemperer, Pollack, and Baehr (3, 5) have suggested that change in the ground substance of the connective tissue as well as in the collagen fibrils contributes to the formation of fibrinoid. Altschuler and Angevine, on the basis of histochemical studies, concluded that the ground substance is the only constant element in the formation of fibrinoid (1) and that the essential feature of the fibrinoid change is the precipitation of the acid mucopolysaccharide of the ground substance by an alkaline protein (1, 2). This suggestion was based upon the finding of a close spatial relationship between fibrinoid and metachromatic staining substances and upon the demonstration, histologically, of positive polysaccharide and arginine staining reactions.

The X-ray pattern of the fibrinoid material in rheumatoid nodules has been recently described as a diffuse ring, quite distinct from that of fibrin and collagen (21). In electron photomicrographs, fibrinoid appeared as an amorphous material. Normal collagen was demonstrated by X-ray pattern and electron microscopic appearance to be in close association with the fibrinoid.

Enzymatic digestion of the fibrinoid in nodules of rheumatic fever has been studied recently by Glynn and Loewi (22). From observations that the fibrinoid in this tissue was resistant to digestion by trypsin over a period of three hours, but was digested by a collagenase preparation from Clostridium Welchii, the authors concluded that fibrinoid is related in structure to collagen.

In order to study the relationship between collagen and fibrinoid, analytical studies of the fibrinoid material of the subcutaneous nodule of rheumatoid arthritis were undertaken. The amino acid, hydroxyproline, constitutes between 13 and 14 per cent of collagen derived from a variety of

[1] This investigation was supported (in part) by a grant from the Masonic Foundation for Medical Research and Human Welfare, and (in part) by research grant No. HG 909 A&R, from the National Heart Institute, of the National Institutes of Health, Public Health Service.

[2] Present address: Department of Chemistry, Meadowbrook Hospital, Nassau County, Long Island, N. Y.

sources (23) and is found in no other protein of the body, except for the small amount present in elastin. Hydroxyproline provides, therefore, a useful means of identifying the presence of collagen in tissue.

The hydroxyproline content of the collagen of the subcutaneous nodule and synovial membrane of rheumatoid arthritis was shown by Bien and Ziff (24) to be the same as that found in purified collagen from a number of normal tissues. Furthermore, the total hydroxyproline content of nodules and of synovial membrane was accounted for completely by the collagen and elastin fractions. It was inferred from this that hydroxyproline was not present in significant amounts in the fibrinoid material of the tissue analyzed.

In the present investigation, previously reported in abstract form (25), fibrinoid has been extracted from rheumatoid nodules by treatment with either dilute alkali or trypsin. The completeness of extraction has been demonstrated by histological section of the extracted tissue. The extracts so obtained have been hydrolyzed and analyzed for hydroxyproline by a sensitive radioisotope chromatographic technique (26), which is described below. The amounts of hydroxyproline found in the extracted fibrinoid were negligible in quantity and of the same magnitude as obtained in extracts of normal skin under the same conditions.

METHODS

The following general procedure was employed; details are given below. Subcutaneous rheumatoid nodules were extracted by two methods which removed the fibrinoid material, and the hydroxyproline content of the fibrinoid-containing extracts was determined. The collagen concentration of the whole nodule tissue was determined by direct analysis. By comparing the relative areas of collagen and fibrinoid on projections of representative stained microscopic sections, the approximate concentration of fibrinoid per gram of wet tissue was calculated. From the hydroxyproline content of the fibrinoid extracts, and the concentration of fibrinoid in the untreated tissue, the maximum percentage of collagen in the fibrinoid was then calculated.

Nodules were obtained from patients with long-standing rheumatoid arthritis, all of them untreated, and with active disease at the time of excision. In one patient, nodules were removed before and after administration of corticotropin. The nodules had been present for periods ranging from one to three years, and all except one, nodule S, were situated below the olecranon process of the forearm. Nodule S was removed from the subcutaneous tissue located over the anterior superior iliac spine. In one nodule which showed cystic changes (nodule V_1), only the solid portions were used for analysis.

Extraction of nodule tissue: One-half to one gram of nodule tissue was minced in a cold chamber to particles about 3 mm. in diameter, weighed, covered with 40 to 60 ml. of either 0.1N or 0.05N sodium hydroxide; or trypsin solution (containing 0.1 mg. of crystalline trypsin [3] per ml. of 0.02M borate buffer in physiological saline at pH 7.75); or borate buffer alone of the same composition, and shaken gently in a water bath at 37° C. for 24 hours at 100 oscillations per minute. An adjacent slice of tissue was taken, as described below, for determination of collagen, and another for histological section. Following extraction of the mince, the supernatant was removed by centrifugation and the residue was washed gently four times with small volumes of physiological saline. The washings were added to the original extract and the resulting solution was diluted to a volume of 100 ml. Total nitrogen was determined by the Kjeldahl method. The residues were then covered with 10 per cent formalin. The final pH of the formalin bath ranged between 6 and 7.

Determination of hydroxyproline: Aliquots of the nodule extracts, containing approximately one milligram of nitrogen, were hydrolyzed with an equal volume of concentrated hydrochloric acid for three hours at 50 pounds. The hydrolysate was then brought to dryness in vacuo, and hydrochloric acid was removed by repeating the evaporation twice after the addition of water. The residue was taken up in one ml. of water while warming on a water bath. An aliquot of this solution, containing approximately 150 mcg. of nitrogen in 0.1 ml., was taken for determination of hydroxyproline by the pipsyl chloride method of Keston, Udenfriend, and Levy (26).

Pipsylation was carried out with 25 mg. of pipsyl (p-iodophenyl sulfonyl) chloride, containing approximately a million counts per micromole of I^{131}, dissolved in 0.6 ml. of half-saturated sodium bicarbonate. A known amount of pipsylhydroxyproline labeled with S^{35}, and having an activity of approximately 10,000 counts per minute, was added. The pipsyl derivatives were extracted with ether and chromatographed on paper for 48 hours at room temperature in n-amyl alcohol saturated with 2N ammonia. The band demonstrating S^{35} radiation was eluted with dilute ammonia, extracted with ether after acidification and re-chromatographed for 12 hours at room temperature in tetrachlorethane saturated with 2N hydrochloric acid. The resulting band, produced by spreading of the pipsylhydroxyproline, was cut into strips, and ratios of S^{35} to I^{131} radiation were determined, using 0.015 inch aluminum foil filter. It was not possible to establish constancy of the ratio of S^{35} to I^{131} in the analysis of nodules V_3 and K because of the small number of iodine counts available in the hydroxyproline bands. The procedure employed, however, was demonstrated in numerous other analyses to achieve separation of pure bands of pipsylhydroxyproline as demonstrated by constancy of S^{35} to I^{131} ratios. The effect of possible contamination of the

[3] Worthington Laboratories, Lot 303.

pipsylhydroxyproline band by other pipsyl compounds containing I^{131} would be to render the values reported maximal ones.

In a recovery experiment in which 18.1 mcg. of hydroxyproline was added to five ml. of a 0.1N sodium hydroxide extract of nodule V_1, and then autoclaved in hydrochloric acid, and analyzed as described above, 17.6 mcg. of hydroxyproline was recovered.

To establish the stability of both bound and free hydroxyproline in alkali under the conditions described, samples of gelatin and l-hydroxyproline were incubated in 0.1N alkali for 24 hours at 37° C. One hundred milligrams of Baker's gelatin was found to contain 9.9 mg. of hydroxyproline when hydrolyzed directly and analyzed by the method of Neuman and Logan (23). After incubation in 100 ml. of 0.1N sodium hydroxide under the conditions described, the hydroxyproline content was unchanged. Similarly when 10.2 mcg. of l-hydroxyproline was incubated under the same conditions, 10.1 mcg. was recovered.

Determination of collagen: The content of collagen was determined on samples weighing between 0.3 and 1.0 Gm. and taken immediately adjacent to the thin slices, described below, from which the microscopic sections for estimation of fibrinoid-collagen ratios were prepared. Collagen was determined by the method of Lowry, Gilligan, and Katersky (27) on the basis of the nitrogen content of the gelatin supernatant assuming 17.5 per cent nitrogen in collagen.

Histological studies: In nodules S, V_1 and V_2, a thin slice about 3 mm. in width was removed from the center of the tissue to be analyzed, and microscopic sections from the above were stained by the van Gieson method for estimation of the relative amounts of fibrinoid and collagen (fibrinoid-collagen ratios) by the following technique: Careful tracings were made of the fibrinoid and collagen areas on lantern slide projections of the stained sections. The tracings representing areas of fibrinoid and collagen were then cut out, and weighed on an analytical balance. The relative weights of the tracings of fibrinoid and collagen were taken as approximations of the relative amounts of these materials in the tissue analyzed. In nodules K and W, insufficient tissue was available for preparation of control sections prior to extraction. It was necessary, therefore, in these nodules to assume the proportion of fibrinoid in two ways: (1) from the histological appearance of serial sections of the extracted tissue, where areas previously occupied by fibrinoid, before extraction, could be identified by the small amounts of this material remaining; and (2) from the gross appearance of serially cut sections of fresh nodule tissue, in which regions of fibrinoid material are identifiable as greyish yellow areas. In these nodules, approximately the same relative amounts of fibrinoid and collagen were present as in nodules S, V_1 and V_2.

Following fixation in 10 per cent formalin, the tissue residues, extracted as described above, as well as the control sections were dehydrated and embedded in paraffin. They were then serially sectioned at 5 microns and stained at intervals with each of the following stains, to establish the completeness of extraction: hematoxylin and eosin, van Gieson, phosphotungstic acid-hematoxylin, trichrome, orcein-picroaniline blue, Millon, toluidine blue, and periodic acid-Schiff. On a number of occasions, the formalin fixing solutions were evaporated to dryness, the residue hydrolyzed and analyzed for hydroxyproline by the method of Neuman and Logan. No hydroxyproline was found.

RESULTS

Extraction of fibrinoid

When minced nodule tissue was extracted with dilute sodium hydroxide solution, examination of serial sections of the extracted residues disclosed that approximately 80 per cent of the fibrinoid material had been removed (Figure 1c), and when the mince was extracted with trypsin solution (Figure 1d) practically all of the fibrinoid had been removed. Extraction of fibrinoid was apparent from the appearance of free spaces devoid of all tissue in the areas previously occupied by fibrinoid material in the control sections (Figure 1a). In these newly formed spaces, strands of intact collagen fibers previously obscured could be seen. Treatment with borate buffer at pH 7.75, extracted relatively small amounts of fibrinoid (Figure 1b).

Analytical data

The concentration of collagen in the nodules analyzed ranged between 70.8 and 159 mg. per gram of fresh tissue (Table I). Fibrinoid concentration, calculated as the product of collagen concentration and fibrinoid-collagen ratio, averaged 29 mg. per gram (range 17 to 40 mg. per gram). From the amounts of hydroxyproline found by analysis of both the sodium hydroxide and trypsin extracts, which varied in different nodules from zero to 0.3 mg. per gram of fresh tissue, the maximum amounts of collagenous material which could be present in the extracted fibrinoid have been calculated. The presence of 13.4 per cent hydroxyproline in collagen (23) has been assumed. The values obtained, averaging 0.67 mg. per gram of tissue (range 0 to 2.20 mg. per gram), appear in all but one instance to be negligible in comparison with the amounts of fibrinoid calculated to be present. The amounts of hydroxyproline found are, moreover, of the

FIG. 1a

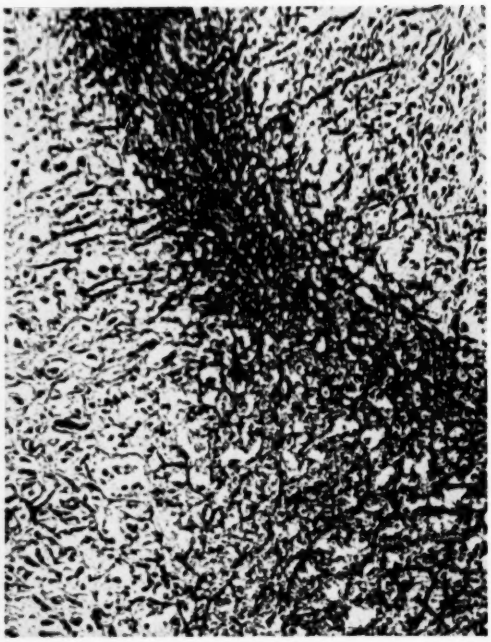


FIG. 1b

FIG. 1c

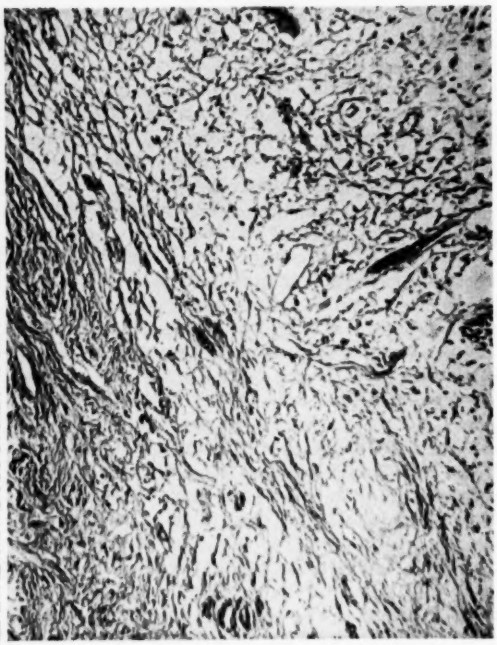


FIG. 1d

TABLE I

*Hydroxyproline content of extracts of subcutaneous nodules**

Nodule	Weight sample (Gm.)	Collagen found (mg./Gm.)	Fibrinoid-collagen ratio measured	Fibrinoid calculated (mg./Gm.)	Extraction medium	Hydroxyproline extractable found† (mg./Gm.)	Collagen extractable with fibrinoid calculated‡ (mg./Gm.)
S	1.01	70.8	0.24	17	NaOH 0.1N	0	0.00
V_1	1.16	104	0.26	27	NaOH 0.1N	0.038	0.28
V_2§	1.27	104	0.26	27	Trypsin	0.30	2.20
K	0.676	135	0.25‖	34	NaOH 0.05N	0.090	0.67
W	0.283	159	0.25‖	40	Trypsin	0.025	0.19

* All analyses are based on wet weight of tissue.

† When 0.627 Gm. of minced human skin was treated with 0.1N NaOH for 24 hours, 0.037 mg. hydroxyproline was extracted per gram of skin. When 0.674 Gm. of skin was similarly treated with a solution of 5 mg. of crystalline trypsin in 40 ml. of 0.02M phosphate buffer at pH 7.75, 0.013 mg. of hydroxyproline was extracted per gram.

‡ This refers to hydroxyproline-containing material extracted with the fibrinoid, which has been calculated as collagen, by multiplying hydroxyproline concentration by the factor 7.46.

§ The patient (V) was treated with corticotropin for 16 days (total dose 3150 mg.) prior to excision of the second nodule.

‖ Values are approximate (see Methods).

same magnitude as found in normal human skin by treatment with dilute alkali or trypsin following the same procedure as applied to the nodules (Table I).

DISCUSSION

Fibrinoid is characteristically found in connective tissue in the "diseases of collagen" (8). Conclusions drawn from investigations of the fibrinoid material of subcutaneous nodules, however must of necessity be limited to this source, since there is no reason to assume that fibrinoid has the same composition in different diseases or even in different tissues. The rheumatoid nodule, moreover, when large enough to yield adequate amounts of tissue for chemical analysis, has usually been present for months to years. Since the composition of fibrinoid in nodules of long standing may differ from that of early nodules, conclusions drawn in the present report are limited by the age of the nodules analyzed, which was one to three years.

Our experiments indicate that the fibrinoid material of the subcutaneous nodule may be almost completely extracted by treatment with dilute alkali or trypsin. The extracts do not contain significant amounts of hydroxyproline. No hydroxyproline was extracted from nodule S, and in three out of four other nodules from untreated patients, the largest concentration extractable was 0.09 mg. per gram of tissue. The small amounts of hydroxyproline extracted were approximately of the same magnitude as were obtained from normal skin by treatment with 0.1N sodium hydroxide and trypsin. A relatively larger amount of hydroxyproline was removed from nodule V_2 on treatment with trypsin. This may perhaps be related to the fact that the patient had been treated with corticotropin for sixteen days prior to ex-

FIG. 1. HISTOLOGICAL SECTIONS OF SUBCUTANEOUS RHEUMATOID NODULES ILLUSTRATING EXTRACTION OF FIBRINOID (NODULE S, TRICHROME STAIN, GREEN WRATTEN B FILTER, MAGNIFIED 120 ×)

a. Control section demonstrating areas of fibrinoid as dense black areas.

b. Section of residues following treatment with 0.02M borate buffer, in physiological saline, pH 7.75, showing removal of small amounts of fibrinoid.

c. Residues following treatment with 0.1N sodium hydroxide, demonstrating clear areas from which fibrinoid has been removed.

d. Residues following treatment with trypsin, in 0.02M borate buffer, pH 7.75, demonstrating clear areas from which fibrinoid has been removed.

cision of the nodule. A total dose of 3150 mg. of corticotropin had been administered.

On the basis of the amounts of hydroxyproline present in the fibrinoid extracts, it is calculated that an average of 0.67 mg. of collagen might have been extracted with approximately 30 mg. of fibrinoid. Collagen could constitute, therefore, about 2 per cent of the weight of fibrinoid. Since the hydroxyproline extracted probably represents the usual complement of this amino acid which is extractable from normal tissue, in view of the recovery of similar concentrations from normal skin, it is likely that little or no collagen is actually present in fibrinoid.

It should be pointed out that the values for the fibrinoid concentration of the unextracted tissue and the hydroxyproline content of the fibrinoid extract are given per gram of wet tissue. The results above, therefore, are independent of the amount of extraneous material extracted with the fibrinoid on treatment with alkali or trypsin. It should also be noted that, although the estimation of fibrinoid is necessarily approximate, appreciable error in this value would not change the overall conclusion because of the relatively small amounts of hydroxyproline recovered.

Though fibrinoid in minced tissue was digested by trypsin, it has been found to be resistant to hydrolysis by Clostridial collagenase (25). The stability of fibrinoid in collagenase solution is not surprising in view of the evidence that it does not contain appreciable amounts of hydroxyproline. Our observations with respect to enzyme digestion differ from those of Glynn and Loewi (22), who analyzed nodules from patients with rheumatic fever. These authors, however, incubated fixed tissue sections for three hour periods, whereas freshly minced tissue was digested for 24 hours in the present experiments.

There is no evidence that fibrinoid in the rheumatoid nodule, as histologically defined, is a homogenous substance. Studies of Kellgren and coworkers (21), using X-ray diffraction and the electron microscope, demonstrated patterns of an amorphous material in intimate contact with normal collagen fibrils. It is reasonable to assume that it is this amorphous material which has been extracted in the present experiments, leaving interspersing apparently normal collagen fibers behind (Figure 1). Our data suggest that degenerated collagen is not present in the amorphous material of fibrinoid unless, of course, the collagen has been so altered in the process of degeneration that the hydroxyproline of the protein has been specifically lost or changed. The selective loss of individual amino acids in the degeneration of proteins finds little precedent, however.

The additional possibility remains, of course, that degeneration of collagen and subsequent removal of the degraded protein precedes the deposition of the fibrinoid material. If this were so, the first step in the fibrinoid change might very well be associated with an alteration of collagen, as suggested by the observations of Klinge (6). It would seem reasonable to assume from the data presented in this paper, however, that if altered collagen is formed, it is hydrolyzed by tissue proteases, and subsequently replaced by fibrinoid, the latter representing merely the end products of inflammation of connective tissue.

SUMMARY

The fibrinoid material of five subcutaneous nodules obtained from patients with rheumatoid arthritis has been extracted by treatment of fresh nodule tissue with either dilute sodium hydroxide or trypsin. The extracts of fibrinoid have been analyzed for hydroxyproline by a sensitive radioisotope derivative technique and found to contain between zero and 0.3 mg. of hydroxyproline per gram of nodule tissue.

Collagen content of the nodule tissue ranged between 70.8 and 159 mg. per gram. From the relative areas of collagen and fibrinoid present in microscopic sections of untreated nodules, the approximate concentration of fibrinoid has been estimated. Values varied between 17 and 40 mg. per gram.

The maximum amounts of collagen which could be present in the extracted fibrinoid, calculated from the hydroxyproline content of the fibrinoid extracts, were found to be about 2 per cent by weight of the fibrinoid. The results indicate that if collagenous material is at all present in the fibrinoid of the subcutaneous nodule, the amounts are negligible.

ACKNOWLEDGMENTS

The authors express their thanks to Drs. Albert Keston and Jean-Claud Dreyfus for their help in the isotopic analytical procedures.

REFERENCES

1. Altschuler, C. H., and Angevine, D. M., Histochemical studies on the pathogenesis of fibrinoid. Am. J. Path., 1949, **25**, 1061.
2. Altschuler, C. H., and Angevine, D. M., Acid mucopolysaccharide in degenerative disease of connective tissue, with special reference to serous inflammation. Am. J. Path., 1951, **27**, 141.
3. Klemperer, P., Pollack, A. D., and Baehr, G., Diffuse collagen disease. Acute disseminated lupus erythematosus and diffuse scleroderma. J. A. M. A., 1942, **119**, 331.
4. Klemperer, P., Diseases of the collagen system. Bull. New York Acad. Med., 1947, **23**, 581.
5. Klemperer, P., The pathogenesis of lupus erythematosus and allied conditions. Ann. Int. Med., 1948, **28**, 1.
6. Klinge, F., Der Rheumatismus; pathologisch-anatomische und experimentell-pathologische Tatsachen und ihre Auswertung für das ärztliche Rheumaproblem. Ergebn. d. allg. Path. u. path. Anat., 1933, **27**, 1.
7. Bennett, G. A., Zeller, J. W., and Bauer, W., Subcutaneous nodules of rheumatoid arthritis and rheumatic fever; a pathologic study. Arch. Path., 1940, **30**, 70.
8. Klemperer, P., Pollack, A. D., and Baehr, G., Pathology of disseminated lupus erythematosus. Arch. Path., 1941, **32**, 569.
9. Masugi, M., and Yä-Shu, Die diffuse Sklerodermie und ihre Gefässveränderung. Virchows Arch. f. path. Anat., 1938, **302**, 39.
10. Pollack, A. D., Visceral and vascular lesions in scleroderma. Arch. Path., 1940, **29**, 859.
11. Gruber, G. B., Zur Frage der Periarteriitis nodosa, mit besonderer Berücksichtigung des Gallenblasen- und Nieren-Beteiligung. Virchows Arch. f. path. Anat., 1925, **258**, 441.
12. Askanazy, M., Über Bau und Entsehung des chron. Magengeschwürs, sowie Soorpilzbefunde in ihm. Virchows Arch. f. path. Anat., 1921, **234**, 111.
13. Grosser, O., Über Fibrin und Fibrinoid in der Placenta. Ztschr. f. Anat. u. Entwicklungsgeschichte, 1925, **76**, 304.
14. Gerlach, W., Studien über hyperergische Entzündung. Virchows Arch. f. path. Anat., 1923, **247**, 294.
15. Wu, T. T., Über Fibrinoidbildung der Haut nach unspezifischer Gewebsschädigung bei der Ratte. Virchows Arch. f. path. Anat., 1937, **300**, 373.
16. Meessen, H., Über Coronarinsuffizienz nach Histamincollaps und nach orthostatischem Collaps. Beitr. z. path. Anat. u. z. allg. Path., 1937, **99**, 329.
17. Henschen, F., Über eine eigenartige, mit Thrombenbildung verbundene Reaktion des Gefässendothels. Acta. med. Scandinav., 1926–27, **65**, 539.
18. Selye, H., The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocrinol., 1946, **6**, 117.
19. Clark, E., Graef, I., and Chasis, H., Thrombosis of the aorta and coronary arteries. Arch. Path., 1936, **22**, 183.
20. Bahrmann, E., Über die fibrinoide Degeneration des Bindegewebes. Virchows Arch. f. path. Anat., 1937, **300**, 342.
21. Kellgren, J. H., Ball, J., Astbury, W. T., Reed, R., and Beighton, E., Biophysical studies of rheumatoid connective tissue. Nature, 1951, **168**, 493.
22. Glynn, L. E., and Loewi, G., Fibrinoid necrosis in rheumatic fever. J. Path. & Bact., 1952, **64**, 329.
23. Neuman, R. E., and Logan, M. A., The determination of hydroxyproline. J. Biol. Chem., 1950, **184**, 299.
24. Bien, E. J., and Ziff, M., Fibrinoid and hydroxyproline of the rheumatoid subcutaneous nodule. Proc. Soc. Exper. Biol. & Med., 1951, **78**, 327.
25. Kantor, T., Sokoloff, L., Smith, A., and Ziff, M., Chemical and histological studies of the fibrinoid material of the subcutaneous rheumatoid nodule. Ann. Rheumat. Dis., 1951, **10**, 471.
26. Keston, A. S., Udenfriend, S., and Levy, M., Determination of organic compounds as isotope derivatives. II. Amino acids by paper chromatographic and indicator techniques. J. Am. Chem. Soc., 1950, **72**, 748.
27. Lowry, O. H., Gilligan, D. R., and Katersky, E. M., The determination of collagen and elastin in tissues, with results obtained in various normal tissues from different species. J. Biol. Chem., 1941, **139**, 795.

THE USE OF RADIOACTIVE CHROMIUM 51 AS AN ERYTHROCYTE TAGGING AGENT FOR THE DETERMINATION OF RED CELL SURVIVAL IN VIVO [1, 2]

By FRANKLIN G. EBAUGH, JR.,[3] CHARLES P. EMERSON, AND JOSEPH F. ROSS
WITH THE TECHNICAL ASSISTANCE OF ROSE ALOIA, PEARL HALPERIN, AND HELEN RICHARDS

(From the Robert Dawson Evans Memorial Laboratory, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine, Boston, Mass.)

(Submitted for publication May 27, 1953; accepted August 18, 1953)

One of the most useful methods available for the quantitative measurement of hemolytic rates in clinical subjects and for the evaluation of red cell viability after storage is based on the survival of transfused erythrocytes. Access to such data, however, has been restricted because of limitations in the methods hithertofore available for measuring red cell survival *in vivo*.

The differential agglutination method of counting donor cells, the procedure most commonly employed, has limitations in that large transfusions are required; the donor blood must be devoid of all antigenic isoagglutinins which are not likewise possessed by the recipient; the recipient's cells must contain agglutinogen A or B or M or a combination of the three agglutinogens which is not contained in the donor blood; and, finally, this method excludes the use of autotransfusion, which would eliminate the risk of transmission of homologous serum jaundice.

The labelling of donor cells with radioactive iron (Fe^{55}) permits the conduct of survival studies on a short term basis (1–3) but re-utilization of radioactive iron released from hemolyzed donor cells and the subsequent incorporation of this label in the recipient's red cells preclude its use in studies extending for periods longer than 24 to 48 hours. Furthermore, this method requires the accumulation in the prospective donor of radioactive iron in relatively high concentration, a procedure which is not desirable. Other tagging methods that have been described, involving the use of sulfhemoglobin (4), N^{15} (5), C^{14} (6), P^{32} (7), and Zn^{65} (8), are similarly objectionable from one or another standpoint.

Gray and Sterling (9, 10) have demonstrated that a firm union between erythrocytes and chromium can be established *in vitro* with great rapidity and with no apparent damage to the exposed cells. These observations imply that radioactive chromium might serve admirably, not only as a tagging device for measuring circulating red cell volume as proposed by these authors, but as a basis for measuring red cell survival *in vivo*. The potential advantages of its use in this manner are several:

1. By reinjecting chromium-tagged red cells into their original host, *i.e.*, autotransfusion, it might be possible to measure the longevity of erythrocytes in their native environment. Such an approach would extend the application of this type of study to hemolytic disorders based on inherent red cell defects which are not amenable to investigation by transfusion, and would insure the validity of survival data in all types of cases.

2. This method may provide a simple and definite method of evaluating the effectiveness of methods and media for *in vitro* preservation of erythrocytes in stored blood. Relatively small samples of donor blood might suffice for accurate counting after injection. A reduction in volume of injected material might be achieved to the extent that several aliquot samples from one unit of blood might be transfused after successive intervals

[1] This work was supported in part by a contract between the Atomic Energy Commission and the Massachusetts Memorial Hospitals, and funds from the American Cancer Society of Massachusetts, Inc., Boston Blood Grouping Laboratory.

[2] Presented in part before the Committee on Blood and Related Problems, National Research Council, Washington, D. C., September 17, 1952, and the American Federation for Clinical Research, Eastern Section, Boston, Massachusetts, April 16, 1953.

[3] Part of this work was performed while a Public Health Service Research Fellow of the National Cancer Institute.

of storage, or after storage under a variety of conditions, affording a more accurate basis for studying the rate of deterioration of preserved red cells.

3. By substituting autotransfusion for transfusions in survival studies, the risk of infection, including hepatitis, and the danger of isoimmunization in the recipient are excluded.

It has been reported (9, 10) that erythrocytes bind 80 to 90 per cent of sodium chromate ($Na_2Cr^{51}O_4$) when trace amounts of the latter are added to cell suspensions *in vitro,* the globin portion of the hemoglobin molecule being the site of the combination. These observations have been amplified and extended by a series of experiments designed to determine:

(1) The effect of storage on the kinetics of chromium uptake by erythrocytes;
(2) the influence of chromium, and the effect of the conditions imposed by the labelling procedure, on the post-transfusion of red cells exposed to this metal *in vitro;*
(3) the avidity and stability of the linkage between erythrocytes and chromium; and
(4) the degree to which sodium chromate released from hemolyzed red cells becomes incorporated into other circulating red cells, or into red cells subsequently produced by the bone marrow of the recipient, the occurrence of which would invalidate red cell survival data obtained by this tagging procedure.

METHODS

Preparation and administration of $Na_2Cr^{51}O_4$ labelled blood

Donor blood used in these experiments was collected and stored in collapsible bags constructed of polyvinyl plastic [4] containing 75 ml. of acid-citrate-dextrose (ACD) solution N. I. H. Formula A.[5] Donor red cells were tagged with radioactive chromium [6] by introducing 3 to 5 ml. of an aqueous solution containing $Na_2Cr^{51}O_4$ (pH 6.0 to 8.0) and with a specific activity of 0.25 to 1.0 millicuries per mg. of chromium metal followed by approximately 10 volumes of isotonic saline into the plastic donor tubing connected to the bag containing whole blood and ACD diluent. $Na_2Cr^{51}O_4$ should not be autoclaved when mixed with ACD solution, since it will decompose under these conditions and become incapable of labelling erythrocytes. The contents of the bag were thoroughly mixed and equilibration of the blood and chromium salt allowed to proceed for various periods of time and under a variety of conditions which are specified in Table I. The bloods of Experiments 1 and 2 were continually agitated on a mechanical shaker during the period of equilibration; the bloods for all the other experiments were gently mixed by inversion every 10 to 20 minutes during the equilibration period. Final preparation of the tagged blood for injection included the preliminary separation of cells and plasma by rotation at 2000 rpm. in an International Centrifuge (Model B. P.) for 30 minutes at room temperature, followed by the removal of approximately 30 to 85 per cent of the plasma. In Experiments 1 and 2 the tagged red cells were washed once in their native plasma. In Experiment 3, the donor cells were suspended in saline, equilibrated with Cr^{51}, washed and finally resuspended in saline before injection. These procedures were calculated to insure the maximum degree of chromium binding with least exposure to damage by the erythrocytes, and reduction to a minimum of the extra-corpuscular chromium in the injected mixture.

The first series of transfusions (Series I, Experiments 1–9) involved the injection of normal donor blood which had been stored at 4 to 6° C. for 0 to 26 days, then tagged with $Na_2Cr^{51}O_4$ by the method described above. Recipients were healthy medical students, each of whom was subjected to a 500 ml. phlebotomy immediately before transfusion. None of these subjects, with one exception, had been transfused previously; (Subject CN had received experimental transfusions on three occasions during the preceding two years).

Individuals receiving experimental transfusions in Series II experiments were hospitalized patients without hematologic abnormalities or diseases known to influence the life span of erythrocytes. In preparation for these experiments, approximately 250 ml. of a 500 ml. ACD blood unit was tagged with Cr^{51} prior to storage. The tagged blood was stored at 4 to 6° C. for periods specified in Table I. The remainder of this unit was stored in the absence of chromium, and the red cells were tagged immediately prior to injection. Fifty ml. aliquots of each portion were transfused at weekly intervals, after the removal of 80 per cent of the plasma, and the resuspension of the cells in a sterile isotonic solution of sodium chloride containing 1 Gm. of dextrose per 100 ml.

As a precaution against bacterial infection of the recipient, stored blood was cultured aerobically and anaerobically at 5° and 37° C. on chopped meat, blood broth, and blood agar, and gram stained smears of the material were examined microscopically prior to injection.

Isoimmunization by transfused red cells was excluded by the observation of a negative Coomb's test, four to five months after transfusion, performed on fresh cells from the original donor following their incubation for one hour at 37° C. with the serum of the recipient.

[4] "Blood Packs" distributed by Fenwal Laboratories, Inc., Framingham, Massachusetts.

[5] Seventy-five ml. of this solution contains trisodium citrate, 1.65 Gm., citric acid, 0.60 Gm., and dextrose, 1.84 Gm.

[6] Obtained from Abbott Laboratories, North Chicago, Illinois. We wish to express our gratitude to Dr. Donalee Tabern for his kind cooperation.

TABLE I

Exp. no.	Donor blood: Preliminary storage in ACD diluent	Donor blood: Blood group	Donor blood: Vol., ml.	Donor blood: Hematocrit, %	Donor blood: Method of chromation: Amount of Cr added, gamma/ml.	Donor blood: Method of chromation: Time of equilibration with Cr^{51}	Donor blood: Method of chromation: Temp., °C.	Donor blood: Method of chromation: Cr^{51} uptake by red blood cell, %	Donor blood: Method of chromation: Proportion of injected Cr^{51} bound to red cells, %*	Recipients: Initials of recipient	Recipients: Diagnosis	Recipients: Blood group	Recipients: Plasma vol., ml.	Recipients: Red cell vol., ml.	Fate of injected material: Time after injection	Fate of injected material: Proportion of injected radioactivity in recipient's plasma, %	Fate of injected material: Per cent donor cell survival: Based on radioactivity of recipient's red cells: Calculated from blood volume	Fate of injected material: Per cent donor cell survival: Based on radioactivity of recipient's red cells: Calculated by extrapolation	Fate of injected material: Per cent donor cell survival: Based on inagglutinable cell counts: Calculated from blood volume	Fate of injected material: Per cent donor cell survival: Based on inagglutinable cell counts: Calculated by extrapolation
Series I—Survival studies by the combined methods of Cr^{51} tagging and selected agglutination counting																				
1	4 hrs.	ON	380	58.0	6.2	3½ hrs.	26–33	91	98	W. C.	Normal subject	AMN	3180	2110	23 min. 19 hrs. 42 hrs. 5 days 110 days	1.6 0.35 0.21	96 101 102 89 2.0	93 97 100 85 2.0	102 94 85 98 2.0	100 92 83 95 2.0
2	4 hrs.	AN	400	48.2	9.3	3½ hrs.	26–33	96	99	J. L.	Normal subject	AMN	—	—	95 min. 18 hrs. 42 hrs. 5 days 105 days	—	—	100 90 98 88 3.7	—	84 100 83 89 3.3
3	7 hrs.	BN	603	48.0	9.5	3½ hrs.	26–27	94	99	C. N.	Normal subject	AB–MN	—	—	24 min. 28 min. 5 days 84 days	—	—	95 100 87 8.2	—	100 99 87 10
4	8 days	ON	510	47.4	2.9	3½ hrs.	4–6	71	85	G. H.	Normal subject	AM	3280	2080	2 min. 44 min. 20 hrs. 2 days 108 days	4.62 3.12 0.73	95 95 101 97 2.7	94 94 100 96 2.7	95 100 86 82 1.7	94 100 86 81 3.8
5	13 days	ON	483	55.2	3.1	3½ hrs.	4–6	68	93	W. A.	Normal subject	OM	3380	2480	6 min. 130 min. 19 hrs. 5 days 102 days	4.9 1.8 0.88	101 95 85 79 3.6	100 94 84 98 3.6	101 87 71 74 7.0	100 86 71 73 5.9
6	15 days	OMN	447	54.8	3.1	3½ hrs.	4–6	68	83	J. H.	Normal subject	AN	3290	2045	22 min. 76 min. 25 hrs. 44 hrs. 119 days	2.7 4.2 .94 .85	102 100 71 72 1.3	100 98 70 70 1.2	102 90 68 66 2.8	100 88 67 65 2.7
7	15 days	OMN	45.0	56.5	35.0	3½ hrs.	4–6	67	89	C. H.	Normal subject	AB	3280	2040	5 min. 100 min. 20 hrs. 45 hrs.	—	104 83 54 54	100 80 52 51	105 89 59 55	100 84 56 53

* After removal of part of the plasma from the original tagged whole blood.

TABLE I—*Continued*

Exp. no.	Preliminary storage in ACD diluent	Donor blood: Blood group	Vol., *ml.*	Hematocrit, %	Method of chromation: Amount of Cr added, *gamma/ml.*	Time of equilibration with Cr^{51}	Temp., °C.	Cr^{51} uptake by red blood cell, %	Proportion of injected Cr^{51} bound to red cells, %*	Recipients: Initials of recipient	Diagnosis	Blood group	Plasma vol., *ml.*	Red cell vol., *ml.*	Fate of injected material: Time after injection	Proportion of injected radioactivity in recipient's plasma, %	Per cent donor cell survival: Based on radioactivity of recipient's red cells: Calculated from blood volume	Based on radioactivity of recipient's red cells: Calculated by extrapolation	Based on inagglutinable cell counts: Calculated from blood volume	Based on inagglutinable cell counts: Calculated by extrapolation
Series I—Survival studies by the combined methods of Cr^{51} tagging and selected agglutination counting—*Continued*																				
8	21 days	AN	376	60.2	3.1	3½ hrs.	4–6	75	95	A. B.	Normal subject	AM	3130	1930	5 min. 132 min. 21 hrs. 26 hrs. 99 days	3.0 1.6 0.42 0.62	94 93 63 66 .74	100 99 67 70 .78	94 85 54 52 1.0	100 89 57 55 1.1
9	26 days	ON	395	60.6	6.8	3½ hrs.	4–6	70	94	H. E.	Normal subject	AN	4140	2830	55 min. 55 min. 130 min. 20 hrs. 24 hrs.	2.7 2.0 1.4 1.5 1.3	89 84 81 55 58	100 95 92 62 66	89 83 80 55 63	100 94 91 62 71
Series II—Survival of aliquots of same unit of blood stored with and without Cr^{51}																				
10	4 hrs.	—	45.9	44.6	1.8	4 hrs.	4–6	73	97	N. C.	Cirrhosis of the liver	—	2100	1580	10 min. 3 hrs. 20 hrs. 45 hrs. 73 hrs.	2.6 0.55	115 111 104 100 101	100 96 91 87 88	—	—
11	7 days	—	45.2	49.0	1.8	7 days	4–6	—	93	P. P.	Diabetes mellitus, mild	—	—	—	35 min. 22 hrs. 29 hrs. 46 hrs. 52 hrs.	—	—	100 89 88 82 88	—	—
12	7 days	—	41.9	45.0	3.4	3½ hrs.	4–6	—	95	C. C.	Rheumatic valvular heart disease, inactive	—	—	—	30 min. 160 min. 28 hrs. 45 hrs. 51 hrs.	—	—	99 96 81 76 75	—	—
13	14 days	—	45.2	30.4	1.8	14 days	4–6	88	95	S. P.	Myocardial infarction, convalescent	—	2750	1885	11 min. 30 min. 6 hrs. 24 hrs. 48 hrs.	2.8	107 104 79 78 76	98 95 72 72 70	—	—

TABLE I—*Continued*

Donor blood										Recipients					Fate of injected material					
					Method of chromation												Per cent donor cell survival			
																	Based on radioactivity of recipient's red cells		Based on inagglutinable cell counts	
Exp. no.	Preliminary storage in ACD diluent	Blood group	Vol., *ml.*	Hematocrit, %	Amount of Cr added, *gamma/ml.*	Time of equilibration with Cr^{51}	Temp., ° C.	Cr^{51} uptake by red blood cell, %	Proportion of injected Cr^{51} bound to red cells, %*	Initials of recipient	Diagnosis	Blood group	Plasma vol., *ml.*	Red cell vol., *ml.*	Time after injection	Proportion of injected radioactivity in recipient's plasma, %	Calculated from blood volume	Calculated by extrapolation	Calculated from blood volume	Calculated by extrapolation
Series II—Survival of aliquots of same unit of blood stored with and without Cr^{51}—*Continued*																				
14	14 days	—	—	30.0	3.0	1 hr.	4–6	28	95	R. R.	Psychoneurosis	—	—	—	22 min. 4 hrs. 22 hrs. 45 hrs. 7 days	—	— 	99 85 77 76 70	—	—
15	22 days	—	45.3	29.0	1.8	22 days	4–6	86	92	B. V.	Diabetes mellitus, mild	—	2370	1734	12 min. 30 min. 6 hrs. 25 hrs. 48 hrs.	—	96 94 63 58 50	99 97 65 60 52	—	—
16	22 days	—	45.2	29.2	3.0	3½ hrs.	4–6	63	89	B. R.	Diabetes mellitus, hypertension and albuminuria	—	2678	1224	20 min. 4 hrs. 23 hrs. 46 hrs. 8 days	2.6	75 53 43 41 35	92 66 53 51 43	—	—
17	29 days	—	45.5	29.8	1.8	29 days	4–6	83	94	L. H.	Cirrhosis of the liver	—	2565	1833	11 min. 30 min. 7 hrs. 24 hrs. 48 hrs.	—	67 54 29 23 23	89 73 39 31 30	—	—
18	29 days	—	45.2	28.0	3.0	3½ hrs.	4–6	—	98	A. M.	Luetic aortic insufficiency	—	—	—	11 min. 20 min. 6 hrs. 23 hrs. 46 hrs.	—	—	89 78 46 31 32	—	—
19	36 days	—	45.2	28.9	1.8	36 days	4–6	83	89	M. J.	Digitalis intoxication, recovered	—	2680	1495	11 min. 28 min. 4 hrs. 25 hrs. 49 hrs.	2.1	54 46 25 17 17	90 77 42 29 27	—	—

Radioactivity measurement

The gamma emission of chromium 51, half life 27.5 days (11), was counted by a well-type scintillation counter,[7] having a gamma counting efficiency of approximately 37 per cent. Counts were performed on 3 to 4 ml. aliquots of whole blood contained in glass test tubes (O. D. 15 mm., or in 4 ml. Gibson hematocrit tubes).[8] When test tubes were used, the contents were always adjusted to the same volume for any series of bloods from the same recipient by the addition of saline solution; Gibson tubes were centrifuged at 3000 rpm. for one hour and, after removal of the plasma, placed in the scintillation counter well. Variations in geometry due to differences in the height of the red cell column in these specimens were compensated for by means of a correction factor applied to the observed counts. This factor was based on the empirical observation that the counting rate decreased 1.25 per cent for each unit of hematocrit increase between 25 and 53 per cent. The number of counts measured in these samples varied from 8000 to 25,600. The background ranged from 100 to 116 counts per minute. Sufficient sample and background counts were measured to assure a standard deviation of 2 per cent or less for all but the weakest samples (Table II). A suitable chromium standard was counted every third or fouth sample tested, all counts being corrected for physical decay of Cr^{51} and for variations in the characteristics of the scintillation counter (which were negligible) by reference to this Cr^{51} standard.

The percentage of Cr^{51} taken up by red cells *in vitro* was determined as follows:

Aliquot portions of whole blood-sodium chromate mixtures, 0.1 to 0.2 ml. in volume, were placed in six test tubes. Three samples were diluted immediately with 10 ml. of isotonic saline, then centrifuged as quickly as possible in order to prevent further Cr^{51} uptake. The red cell sediment in these tubes was washed three times and suspended in isotonic saline solution. The radioactivity in each tube was measured and the percentage uptake of chromium by the red cells calculated by dividing the radioactive count in each sample of washed erythrocytes by the total number of counts in an aliquot mixture of whole blood and chromium, and multiplying the resulting value by 100. The concentration of Cr^{51} in the red cells of the recipient was obtained by dividing the number of counts observed in each sample by its volume, expressed as ml., and by its hematocrit reading. The hematocrit readings were not corrected for amount of "trapped" plasma for reasons detailed below.

TABLE II

*Comparison of the theoretical counting errors of the selective agglutination and Cr^{51} methods**

Days after transfusion	Percentage donor cells surviving destruction	
	Selective agglutination	Cr^{51} counting
0	100 ± 12	100 ± 4.0
30	64 ± 7.7	57 ± 2.3
86	14 ± 2.2	8.0 ± 0.32
103	4.0 ± 1.2	3.0 ± 0.24

* These figures represent errors resulting solely from random distribution, their magnitude depending upon the number of units counted and the ratio of the numerical value of the sample to that of the blank or background. The standard deviation is expressed as per cent of the original number of donor cells surviving destruction on the day indicated. The standard deviation expressed as per cent of donor cells present on a given day would be 12 per cent and 4 per cent for the selective agglutination technic and Cr^{51} technic, respectively, on day 0 and 28 per cent and 8 per cent, respectively, 103 days after transfusion.

[7] Sodium iodine thallium activated crystal in an Atomic Instrument Company scintillation head.

[8] Macalaster Bicknell Company, Boston, Massachusetts.

Blood volume measurement

The plasma volume of each recipient was determined by the T-1824 dye dilution technique (12). The total circulating red cell mass was calculated on the basis of the plasma volume and venous hematocrit, the latter having been modified by the correction factor 0.9 to correct for plasma occluded in the packed cell mass or for inequalities between the hematocrit of large and small vessels or for a combination of both.

(1) red cell volume (ml.)

$$= \frac{\text{plasma volume (ml.)}}{100 - 0.9 \times \text{venous hematocrit (\%)}} \times 100 - \text{plasma volume (ml.)}$$

In the first series of experiments red cell volume was determined in this manner two to three months following transfusion; in Series II, this was done at the time of injection of Cr^{51} tagged cells.

Calculation of post-transfusion survival of chromium-labelled erythrocytes

The percentage survival of transfused Cr^{51} labelled cells in the recipient's circulating blood at any time after the transfusion was calculated from the following formula:

(2) Percentage donor cell survival

$$= \frac{\text{Radioactive } Cr^{51} \text{ per ml. of recipient's packed red blood cells} \times \text{recipient's total red cell mass}}{\text{Total erythrocyte } Cr^{51} \text{ transfused}^{9}}$$

The concentration of Cr^{51} per ml. of the recipient's packed red blood cells (the "specific activity of the red cells") was calculated by dividing the radioactivity per ml. of whole blood by the uncorrected centrifuge venous hematocrit value as stated above. No correction factor for "trapped" plasma in the cell mass was applied to this hematocrit value because of disagreement on the part of the authors as to the magnitude of this factor (13, 14). If the hematocrit value had been modified by such a factor, the specific activity of the red cells and hence the percentage survival would have been higher than the values reported in this study, the difference being equal to the value of the correction factor applied.

[9] Note: this value does not include the small amount of free Cr^{51} which was present in the injected plasma.

This method of calculating percentage survival is based exclusively upon measured values, *i.e.*, the total radioactivity of the tagged donor cells, the blood volume of the recipient, and the actual concentration of transfused cells per unit volume of the recipient's blood. (See Table I.)

Inagglutinable cell counts

Red cell counts were performed on cell suspensions prepared by diluting 0.1 ml. samples of whole blood with 20 ml. of isotonic saline solution. A 0.1 to 0.2 ml. aliquot sample of each suspension was placed in a 12 by 75 mm. test tube containing approximately 10 mg. of dried anti-A or M serum [10] (15). A second aliquot sample was placed in a hemocytometer chamber, and the total red cell concentration determined on the basis of a count of 1000 cells or more. The test tubes containing the cell suspension and dissolved antisera were centrifuged at 900 rpm. for one minute, after which the cell sediment was resuspended by tapping the tube vigorously 30 to 50 times. An aliquot portion of this suspension containing a mixture of agglutinated and inagglutinated cells was then transferred to a counting chamber and the free, inagglutinated cells counted. In each instance the minimum number of inagglutinated cells counted was 200, or the number present in 18 $mm.^2$ of counting area when the number in this area was less than 200. Prior to each transfusion, a blank inagglutinable cell count was performed on the recipient cell suspension containing an aliquot portion of the anti-serum reserved for that particular study. The ratio of the number of inagglutinable cells minus the blank count to the total cell count multiplied by 100 was expressed as per cent donor cells. The field error in the selective agglutination counts reported in this series varied from a S. D. ± 6 to S. D. ± 15 per cent (Table II). Calculations of percentage of survival of donor cells counted by the selective agglutination method were based on T-1824 blood volume data, as in the case of the Cr^{51} tagged cells.

EXPERIMENTAL OBSERVATIONS

Chromation of Erythrocytes in Vitro

Influence of temperature and pH

The rate at which chromium combined with red cells when sodium chromate was added to whole ACD blood was dependent upon the temperature and pH of the mixture, as demonstrated in Figure 1 and Table III. At 39° C., 90 per cent of available chromium was incorporated in the erythrocytes within a period of five minutes, as compared to a 50 to 75 per cent uptake at 26° C. and 10 to 20 per cent at 1.8° C. After thirty minutes of equilibration the proportions taken up at 26° and 39° C. were comparable, *i.e.*, approximately 90 per cent. The influence of pH on the kinetics of this reaction is apparent from the observation that the chromium uptake at pH 6.0 was three to four times as rapid as at pH 7.3 (Table III).

Influence of storage, concentration of $Na_2Cr^{51}O_4$, and stability of the Cr^{51}-erythrocyte union in vitro

Preliminary storage of erythrocytes at 4° to 6° C. for periods as long as twenty-nine days did not appear to reduce their affinity for chromium. On the contrary, Cr^{51} uptake was more rapid in stored than in fresh blood, due perhaps to the greater acidity of the former. The pH of whole ACD blood immediately after collection is approximately 7.2; after storage for one week at 4° to 6° C. the expected pH value is approximately 7.0, falling to 6.8 after two weeks, and 6.6 to 6.7 after three weeks or longer. Chromium uptake, expressed in terms of the percentage bound by red cells following the addition of $Na_2Cr^{51}O_4$, was not influenced by variations in concentration ranging from 0.25 to 9.5 micrograms of chromium salt (expressed in terms of chromium metal content) per ml. of whole blood (Table I, Figure 1). The erythrocyte-chromium bond remained stable during storage in ACD plasma, inasmuch as there was no elution of Cr^{51} from tagged erythrocytes stored as long as thirty-six days (Table I, Experiments 10, 13, 17, and 19).

Does Cr^{51} released from hemolyzed cells relabel the recipient's red cells?

The extent to which Cr^{51} from hemolyzed red cells may combine with intact erythrocytes *in vitro* was investigated as follows:

Radioactive sodium chromate was added to whole ACD blood in a concentration of 2.5 micrograms per ml., and the mixture, divided into three aliquot samples, was allowed to stand for ninety minutes at 1.8°, 26.6°, and 39° C. The red cells in each sample, which had taken up 40, 84, and 92 per cent of available chromium, respectively, were washed three times in saline solution and then subjected to repeated freezing and thawing until hemolysis was complete. Each hemolysate was mixed with an equal volume of washed, intact erythrocytes and incubated for ninety minutes at

[10] Powdered grouping serum, Lederle Laboratories, Pearl River, New York.

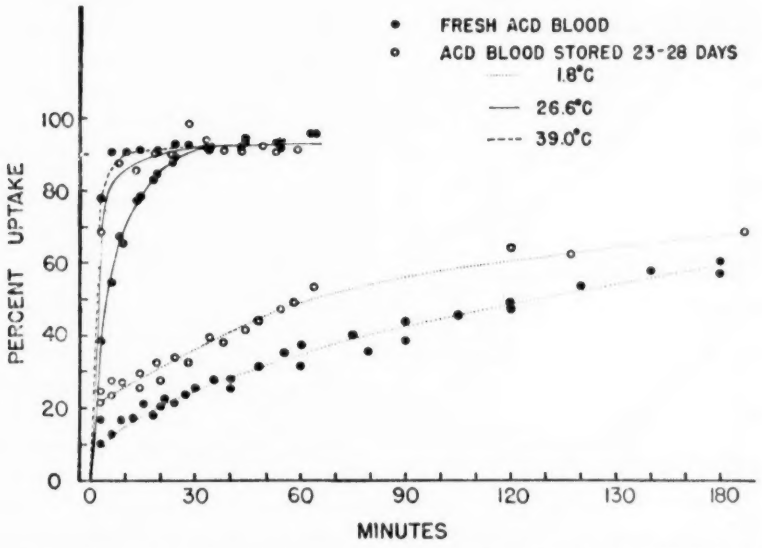


FIG. 1. THE RATE OF SODIUM CHROMATE UPTAKE BY FRESH AND AGED ACD BLOOD AT DIFFERENT TEMPERATURES

The pH of the stored blood was between 6.7 and 6.8. The pH of the fresh blood was 7.1 to 7.2. The concentration of $Na_2Cr^{51}O_4$ (expressed in terms of chromium metal content) was 2.5 gamma per ml. in all bloods except one of the two fresh bloods studied at 26° C. which was 0.25 gamma per ml.

the same temperature to which the hemolyzed cells had originally been exposed. Finally, the intact cells were separated, washed, and tested for Cr^{51} uptake, which was discovered to be negligible in all samples (*i.e.*, less than 0.5 per cent). The *in vitro* observations were substantiated by the following *in vivo* experiment: Tagged erythrocytes were washed three times in saline and lysed by the addition of ten volumes of distilled water. The hemolysate was then made isotomic by the addition of NaCl and passed through a Berkefeld filter and injected back into the original donor of the red cells. During the first twenty-four hours 27 per cent of the injected dose was excreted in the urine. None of the injected Cr^{51} was fixed to, nor reappeared in, the recipient's cells during the thirty-day observation period following the injection.

TABLE III

*Effect of pH on the rate of Cr^{51} uptake by aliquot samples of fresh erythrocytes in whole ACD blood at 2° C.**

The concentration of $Na_2Cr^{51}O_4$ was 2.5 gamma per ml. whole blood

pH	Per cent Cr^{51} uptake by erythrocytes	
	After 3 min. equilibration	After 15 min. equilibration
	%	%
7.3	8	11
6.9	11	12
6.0	28	41

* Effect of pH on the rate of Cr^{51} uptake by red cells at 2° C. after addition of 2.5 gamma of chromium (as $Na_2Cr^{51}O_4$) per ml. of fresh whole ACD blood.

Survival of chromated erythrocytes in vivo

The survival of Cr^{51}-labelled donor erythrocytes has been observed in 19 subjects based on the radioactivity of the recipient's blood after transfusion. The results of these studies, in part, are presented in Table I, together with data pertaining to the preliminary processing, storage, and chromation of injected cells. Nine recipients were studied by the combined application of the chromium labelling and selective agglutination counting methods, the results of which are likewise included in Table I (Experiments 1 to 9), and represented in graphic form in Figure 2. The survival in five recipients of transfused donor cells from the same half unit

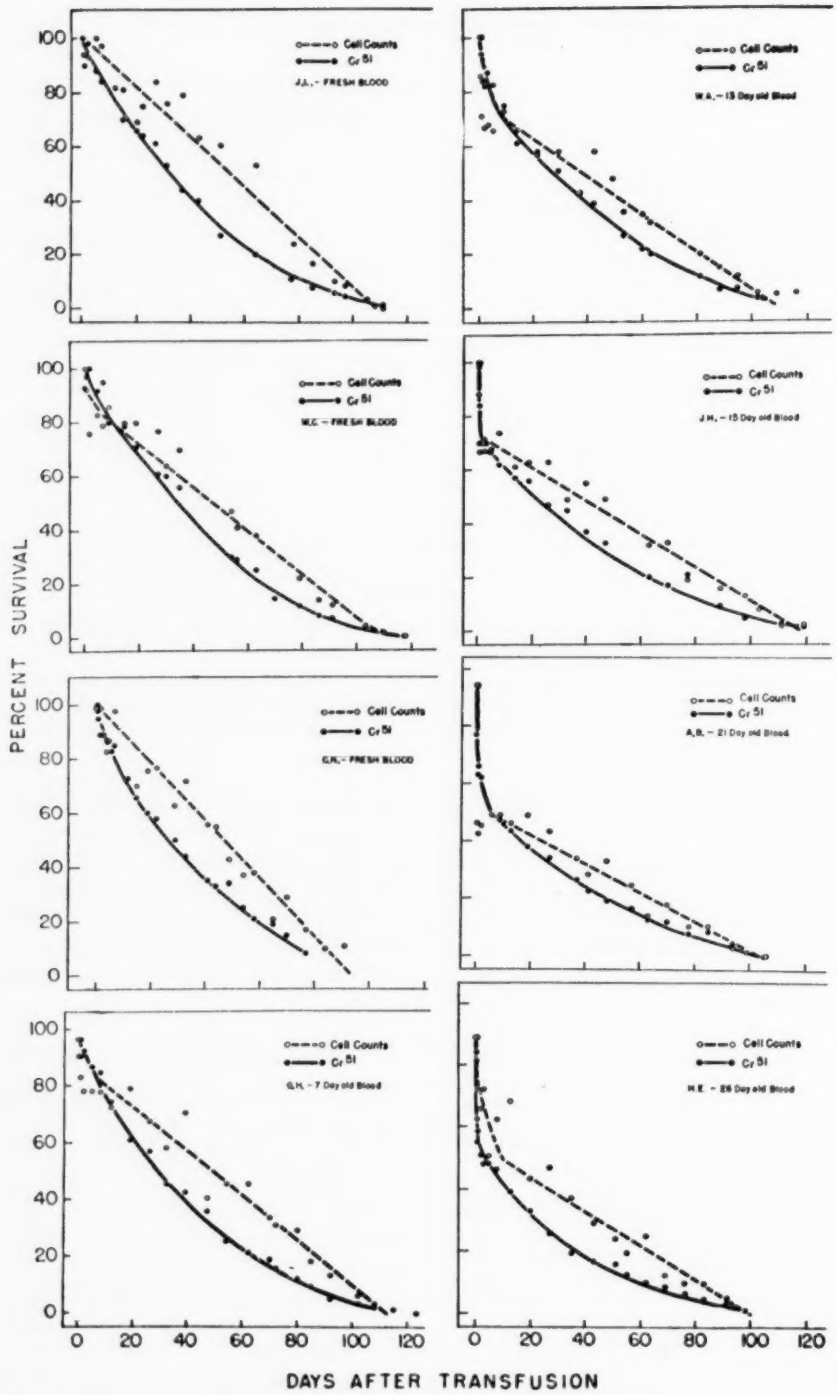

PERCENT SURVIVAL
DAYS AFTER TRANSFUSION
Cell Counts
Cr 51
J.L. - FRESH BLOOD
W.A. - 13 Day old Blood
W.C. - FRESH BLOOD
J.H. - 15 Day old Blood
G.H. - FRESH BLOOD
A.B. - 21 Day old Blood
G.H. - 7 Day old Blood
H.E. - 28 Day old Blood

FIG. 2. COMPARISON OF RED CELL SURVIVAL DATA OBTAINED BY SELECTIVE AGGLUTINATION AND Cr^{51} COUNTING

of donor blood, stored with $Na_2Cr^{51}O_4$ for periods varying from one to five weeks, is shown in Table I and Figure 5. As a control on the effect of storing red cells with $Na_2Cr^{51}O_4$, the survival of aliquot portions from the other half of the same unit of blood which were tagged just before their transfusion is shown in Table I (Experiments 10, 12, 14, 16, and 18) and Figure 5.

Comparison of red cell survival as measured simultaneously by the chromium technique and selective agglutination

The survival of Cr^{51}-labelled erythrocytes measured in the same recipient both by radioactive and selective agglutination counting is represented by the paired curves in Figure 2, constructed from data obtained by both techniques on twenty or more occasions over a period of 100 to 130 days following each transfusion of Cr^{51}-labelled red cells. Donor cell survival as measured by the Cr^{51} method is represented by a complex curve which is neither logarithmic nor linear, and the end point of which coincides with the total disappearance of inagglutinable cells.

Comparison of data obtained by selective agglutination and Cr^{51} counting during the first two days (Figure 3) indicates that the former values are consistently higher during the first forty-eight hour period following transfusion, the degree of difference averaging 6.7 per cent. The probability that this difference was due to chance is less than 0.01 per cent. The discrepancy may be due to a systematic error in the Cr^{51} and in selective agglutination cell survival data. It is unlikely that it is due to tagging of the recipient's erythrocytes by the small amount of injected unbound $Na_2Cr^{51}O_4$ (average of 6.5 per cent of the total injected radioactivity for Experiments 1 to 9, Table I) since only about 10 per cent of free $Na_2Cr^{51}O_4$ when injected intravenously tags recipient erythrocytes as was demonstrated in three experiments of which the following experiment is an example:

A solution of sodium chromate containing a total of 0.8 mg. of $Na_2Cr^{51}O_4$ expressed in terms of its chromium metal content was added to 100 ml. of saline and the mixture injected intravenously. Five minutes following injection 12 per cent of the injected Cr^{51} was bound to the erythrocytes and remained fixed at that level in the erythrocytes

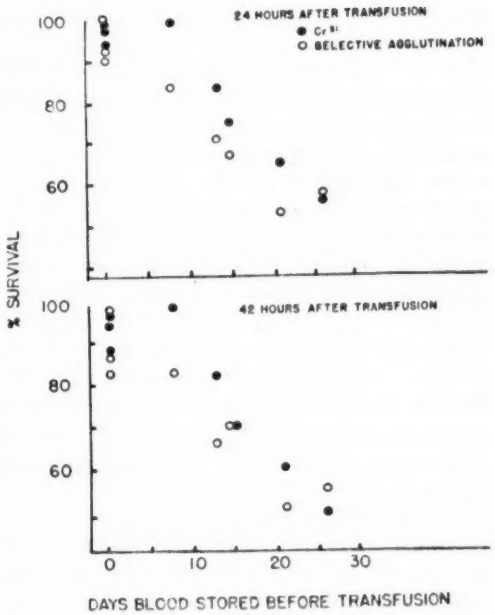


FIG. 3. COMPARISON OF SELECTIVE AGGLUTINATION AND Cr^{51} COUNTS AS A MEASURE OF SURVIVAL ON THE SAME UNIT OF BLOOD 24 AND 42 HOURS AFTER TRANSFUSION

for a period of at least one week, after which a gradual decline in chromium concentration occurred. It may be pointed out that the amount of chromium injected in this experiment was greater than the largest amount of unbound chromium injected in the transfusion experiments described in this report.

All Cr^{51} values obtained one week or more following transfusion are consistently lower than the corresponding values obtained by selective agglutination counts (Figure 2). The difference between these two curves might be explained either on the basis of a continuous chromium elution from the circulating labelled cells or on the assumption that the amount of chromium taken up by the donor cells is not the same for each cell, but in direct proportion to the age of the red cell at the time of collection from the recipient, *i.e.*, the senescent cells binding the most Cr^{51}, and the young cells the least. There is no experimental data for or against either hypothesis at present. However, for purposes of discussion, the progressive decline in the concentration of Cr^{51} in circulating donor cells will be characterized as an elution phenome-

non. The rate of Cr^{51} loss from intact donor cells (*i.e.*, biologic decay), as distinguished from loss of the Cr^{51} attributable to the hemolysis of tagged cells, may be calculated on the basis of changes in the ratio of cell survival determined by Cr^{51} counts and the corresponding values based on selective agglutination counts. A semilogarithmic plot of the per cent of original amount of Cr^{51} remaining on the donor cells for the transfusion experiments (Figure 4), indicates that the biologic half-life of red cell bound Cr^{51} may range from fifty-one to ninety days (Table IV). Excluding Experiment 9 from these calculations, the results of which vary more than two standard deviations from the mean of all the data, the mean half-life of chromium elution is 77 ± 12. Survival of transfused erythrocytes can be accurately measured during the first two days after transfusion without corrections for this loss of Cr^{51} since less than 3 per cent of transfused donor cell Cr^{51} is eluted from the red cells during this interval. Long-term survival values based on Cr^{51} determinations are subject to error unless the phenomenon of chromium elution is taken into account and a correction factor introduced to compensate for its effect. Such a correction factor may be based on the observed half-life of chromium elution, or biological decay of Cr^{51}. For example, 66 per cent of injected cell-count Cr^{51} is present in the recipient (Experiment 2, Table I, and Figure 2) on the twentieth day and 53 per cent on the thirtieth day following transfusion; the average rate of donor cell hemolysis throughout the ten-day period, based on uncorrected data, would be $(66 - 53)/10$ or 1.3 per cent per day for the ten-day period. However, only 83 per cent of the original amount of Cr^{51} present in the transfused cells remains twenty days after transfusion and 76 per cent thirty days after transfusion. Correcting for this "elution" of Cr^{51} by use of the above figures, the hemolytic rate would then equal $(80 - 70)/10$ or 1.0 ± 0.15 per cent as calculated from the Cr^{51} data in contrast to the observed hemolytic rate of 0.9 per cent per day as determined by selective agglutination counting for the same recipient during the same interval of time. A large part of the discrepancy between the two methods is due to the variation in Cr^{51} elutions observed in donor blood thus far studied.

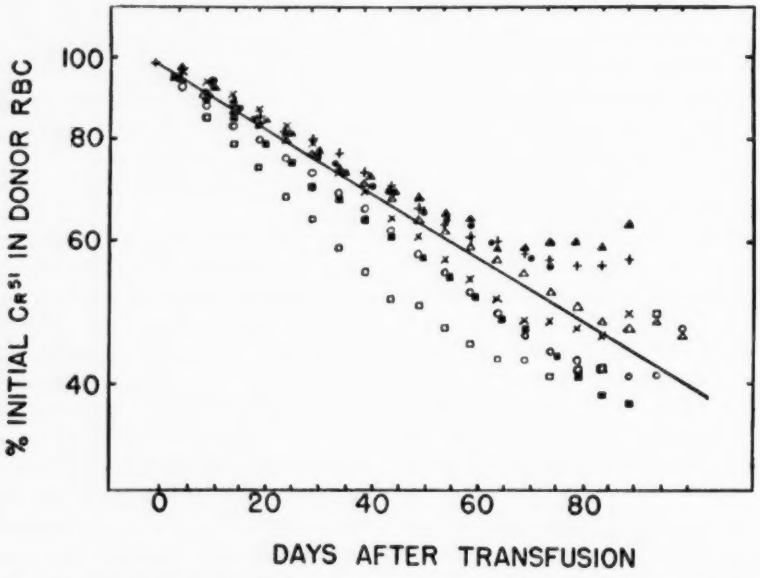


FIG. 4. THE RATE OF Cr^{51} ELUTION FROM TRANSFUSED Cr^{51} LABELLED ERYTHROCYTES

The ordinate scale is logarithmic and indicates the percentage of original Cr^{51} present in the circulating red cells for any given day (abscissa) after transfusion. The individual experiments are identified by the following symbols: Exp. 1—×; Exp. 2—○; Exp. 3—●; Exp. 4—■; Exp. 5—+; Exp. 6—△; Exp. 8—▲; Exp. 9—□.

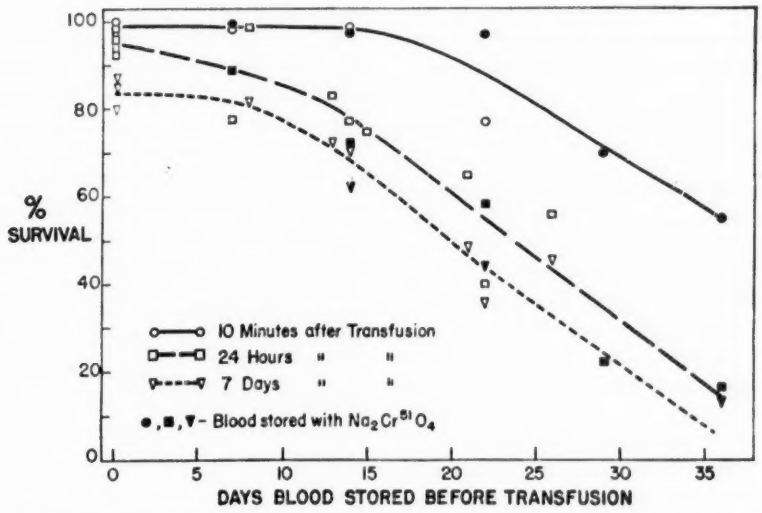


FIG. 5. THE SURVIVAL OF RED CELLS AS MEASURED BY COUNTING Cr^{51} TEN MINUTES, TWENTY-FOUR HOURS, AND ONE WEEK AFTER TRANSFUSION

The closed symbols represent blood stored with $Na_2Cr^{51}O_4$, the open symbols blood stored without $Na_2Cr^{51}O_4$.

The Effect of Chromation on Red Cell Survival

The maximum red cell survival of fresh ACD blood is commonly stated to be approximately 120 days. Inspection of the curves in Figure 2 based on selective agglutination counts indicates that the extinction point of Cr^{51} counts following transfusion of blood previously stored 0 to 8 days range from 108 to 115 days. The sole exception was found in Experiment 3 in which the end point occurred at ninety-four days. Inasmuch as the subject of this experiment had previously received three experimental transfusions, it may be speculated that the relatively brief survival period observed in this study may have been attributable to isoimmunization with the production of isoantibodies of an obscure variety or so weak as to escape detection by the indirect Coomb's test. Also, the donor blood used in this experiment was washed twice in isotonic saline before injection, a procedure which may conceivably have damaged the erythrocytes.

TABLE IV

Rate of chromium 51 elution from circulating donor erythrocytes

Experiment	Preliminary storage of donor blood	Half-life of chromium in red cells *days*
1	4 hrs.	64
2	4 hrs.	65
3	7 hrs.	90
4	8 days	65
5	13 days	87
6	15 days	82
8	21 days	87
9	26 days	51
	Mean ± S.D.	74 ± 33 days
	Mean (excluding Exp. 9) ± S.D.	77 ± 12 days

Effect of erythrocyte storage with $Na_2Cr^{51}O_4$ on survival

The effect on red cells of prolonged exposure to chromium, as evidenced by their post-transfusion survival, was studied in Experiments 10 to 19 (Table I), which were conducted as follows:

Two equal portions of fresh ACD blood from the same unit of blood were placed in separate containers. To one of these was added $Na_2Cr^{51}O_4$. Both portions were placed in storage. At weekly intervals, 50 ml. samples from each portion were removed and transfused, the portion which had been stored without chromium having been tagged immediately before injection. Comparison of the survival of blood stored with and without chromium (Figure 5) indicates that the portion ex-

posed to chromium continuously throughout storage was entirely comparable to the control samples labelled immediately before transfusion.

Effect of red cell exposure to high concentrations of $Na_2Cr^{51}O_4$

Exposure of red blood cells to sodium chromate within the concentration range of 1.8 to 9.5 micrograms per ml. of whole blood (expressed as elemental chromium) in four fresh blood samples had no apparent influence on immediate post-transfusion survival (Table I, Figure 2, Figure 5).

In order to determine the effect of relatively high concentrations of chromium on red cell longevity, an experiment was performed involving the transfusion of two aliquot samples from the same unit of donor blood which had previously been stored at 4 to 6° C. for fourteen days, then labelled with $Na_2Cr^{51}O_4$. The proportions of blood and sodium chromate were such that the concentrations of chromium metal in the two samples differed by a factor of 10, *i.e.*, 3.1 micrograms as compared to 35 micrograms of chromium metal per ml. of whole donor blood. Inspection of the curves representing the survival of these two portions (Figure 6) indicates that within twenty-four hours following transfusion 50 per cent of the heavily tagged blood was eliminated, in contrast to 30 per cent destruction in the case of the lightly exposed cells. The results of this single experiment are by no means conclusive, in view of the fact that these observations were made in different recipients, but suggest that exposure to chromium in a concentration of 35 micrograms per ml. of whole blood may have resulted in cell damage evidenced by an increase in the proportion destroyed immediately following injection. Throughout the remainder of their life span, both the cells which had been exposed to the high concentration and those which were exposed to the low concentration of chromium were eliminated at rates that were equal to those observed for unlabelled donor blood stored in ACD plasma for the same period of time.

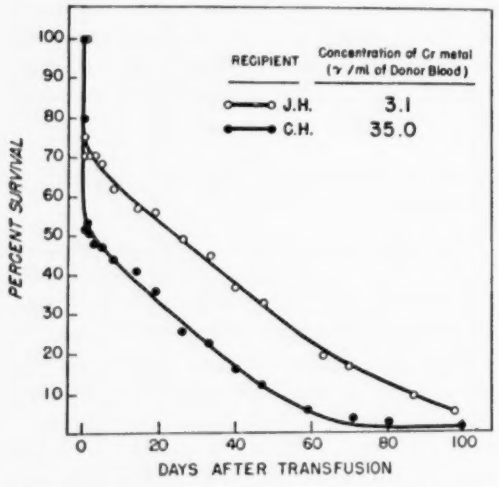


FIG. 6. COMPARISON OF THE SURVIVAL, AS MEASURED BY COUNTING Cr^{51}, OF TWO ALIQUOTS OF THE SAME DONOR BLOOD, ONE OF WHICH WAS TAGGED WITH A CONCENTRATION OF 35 GAMMA, AND THE OTHER 3.1 GAMMA OF $Na_2Cr^{51}O_4$ (IN TERMS OF CHROMIUM METAL PER ML. OF WHOLE BLOOD)

Excretion of Cr^{51}

The excretion of Cr^{51} in the urine at various intervals after transfusion is shown in Table V. The data indicate that for long periods of time after the transfusion at least a quarter of the amount of Cr^{51} known to have been removed from the circulation is excreted in the urine. Exceptions occurred during the period of rapid hemolysis in the case of Experiments 9 and 15, where only 8.5 per cent and 17 per cent, respectively, of the amount disappearing from the circulation during the first twenty-four and forty-eight hours appeared in the urine. Preliminary observations in dogs show that during the interval of 0 to 7 days after a

TABLE V

Urinary excretion of chromium 51 compared to the amount disappearing concomitantly from the circulation in eight transfusion recipients

Experiment no.	Days after transfusion	Per cent of total injected dose of Cr^{51} disappearing from circulating blood during this period	Per cent of Cr^{51} disappearing from the circulation excreted in the urine	Per cent of total Cr^{51} excreted in urine
10	0–2	11.0	52	5.7
8	0–1	29	45	13.1
15	0–1	46	8.5	3.9
9	0–2	64	17	10.9
8	12–13	1.07	93	0.99
4	28–29	0.97	102	0.99
6	30–31	0.70	27	0.19
7	30–31	0.84	24	0.20
1	57–58	0.72	65	0.47

transfusion of poorly preserved blood, 85 per cent of which was removed from the circulation during the first week post-transfusion, 20 per cent of the injected dose was excreted in the urine and 7 per cent in the feces.

DISCUSSION

The experimental evidence herewith presented indicated that radioactive, chromium-labelled erythrocytes can be employed for evaluating the post-transfusion survival of red blood cells for periods of at least twenty-four to forty-eight hours after transfusion and possibly for much longer periods of time, perhaps for their entire life span. The method is ideally suited for evaluating the effectiveness of *in vitro* erythrocyte preservation techniques, and possibly may prove to be of great value for studying the life span of erythrocytes in various disease states.

The considerations which are of paramount importance in establishing the validity and applicability of this method are the following:

(1) The capacity of erythrocytes to bind the chromate ion under certain conditions;
(2) The "firmness" or permanency of this binding;
(3) The possibility that the labelling of erythrocytes with radioactive chromium adversely influences the survival of the cells in the recipient;
(4) The possibility that radioactive chromium liberated from transfused red cells (either by elution or from cellular destruction) may re-enter and tag the recipient's own cells;
(5) The question as to the safety of this method from the standpoint of radiation effects in human subjects receiving blood labelled with radioactive chromium.

Erythrocyte-chromium union

The union between erythrocytes and radioactive sodium chromate takes place *in vitro* regardless of the duration of time that the erythrocytes have been stored prior to their exposure to sodium chromate. This fact makes possible the labelling and subsequent post-transfusion study of blood which has been stored for prolonged periods of time before injection. It is also apparent from our studies that the chromation of fresh blood and storage in the chromated state is in no way different from the standpoint of eventual post-transfusion survival than chromation of blood just prior to transfusion. These features make the method eminently satisfactory for a study of blood storage methods.

It is quite feasible to chromate erythrocytes at refrigeration temperatures without subjecting them to the possibly deleterious influence of incubation, since our studies show that erythrocytes will take up chromium at 4° C. in quantities sufficient to allow transfusion studies. The rapidity and amount of chromium taken up by red cells increases with increase of temperature to 39° C.

Chromation proceeds slightly more rapidly and to a somewhat greater degree at a pH of 6.0 than at a pH of 7.2, which is reflected in a greater uptake of chromium by blood stored for significant periods of time than by fresh blood, since the pH of stored blood is lower than that of fresh blood.

The percentage of sodium chromate bound to erythrocytes is not appreciably influenced by the amount of chromate ion present within the range of 0.25 to 10.0 micrograms of chromium ion per ml. of blood.

Our studies do not provide information as to the nature of the chromate-erythrocyte bond—whether it be simple physical adsorption, chemical linkage, or enzymatic binding.

The erythrocyte-chromate linkage appears to be quite stable, at least *in vitro*, inasmuch as repeated washings with saline removed negligible amounts of the labelling material. *In vivo*, however, there is a steady and apparently exponential disappearance of the radioactive chromium from the labelled erythrocytes, half of the chromium disappearing in 77 ± 12 days in our experiments. Such a disappearance might be explained either by a process of elution of the chromate ion from the erythrocyte, or by preferential rapid destruction of the chromated cells. The rather wide range of biologic half times may well be accounted for by the fact that the bloods on which these elution rates were determined had been processed in a variety of ways. It may be that freshly drawn and chromated bloods reinfused into a recipient may have a more nearly constant rate of elution.

Is there labelling of the recipient's erythrocytes by radioactive chromium liberated in vivo?

This is a critical consideration from the standpoint of the validity of this method for evaluating long-term erythrocyte survival. Thus, if chromate ion which has escaped from intact erythrocytes or is released by hemolysis from senescent red cells were to re-enter and label the erythrocytes of the recipient, the rate of the disappearance from the circulating cell mass of the recipient could not be interpreted in terms of the survival of donor cell population.

Analysis of the cases in which as much as fifty per cent of the transfused, labelled cells were destroyed during the first twenty-four hours after transfusion revealed that survival, as measured simultaneously by selective agglutination and Cr^{51} methods, agreed closely following this period of rapid destruction. If retagging of the recipient's red cells had occurred under these circumstances, the survival as measured by Cr^{51} would have been significantly higher than that as determined by selective agglutination. The above observation does not exclude re-utilization of the injected Cr^{51} by the recipient's bone marrow and the gradual incorporation of Cr^{51} in recipient red cells. This possibility is excluded by the observation that after the injection of Cr^{51}-tagged hemoglobin, none appeared in the recipient's red cells at any time during the following month.

Since from 1 to 17 per cent of the injected chromate was not bound to the donor cells employed in this study, but was present in the donor plasma, the possibility existed that recipient cells could have been tagged by this "free" chromate. Experiments have shown that no more than 12 per cent of the injected aqueous chromate is attached to the recipient's red cells. Thus, no more than 12 per cent of the total amount of injected Cr^{51} contained in the donor plasma could have become bound to the recipient's cells. This observation has practical as well as theoretical importance, since it makes unnecessary the processing of blood with the aim of complete removal of the portion of added sodium chromate which does not become fixed to the red cells.

The in vivo survival of chromated erythrocytes

Careful determination of the survival of chromated erythrocytes by the Ashby differential agglutination method indicates that during the first twenty-four to forty-eight hours after transfusion, the behaviour of chromated cells is quite comparable to the survival which would be expected of non-chromated red cells. This is true of fresh bloods in which survival of the transfused cells is normal, and of bloods stored for prolonged periods of time in which there is initial rapid destruction of the donor cells. These findings establish the validity of the chromated cell method of evaluating the effectiveness of blood preservation methods, since it is the survival of donor cells in the immediate post-transfusion period which is of paramount importance in evaluating the viability of transfused erythrocytes.

The survival values of the bloods stored for prolonged periods of time in ACD medium as indicated by the chromated cell method are lower by some 10 per cent than the values previously reported as determined by the radio-iron tagged cell method (1–3). This discrepancy might be explained by the fact that the hematocrit value employed in calculating red cell survival in these studies was not modified by a correction factor as was done in the studies on survival of radio-iron tagged cells. It might also reflect a deleterious influence of sodium chromate on the erythrocytes. The concentration of chromium which is definitely injurious to erythrocytes has not been determined in this study. Suggestive evidence was obtained which indicated that a concentration of 35 micrograms of sodium chromate (expressed as elemental chromium) per ml. of whole blood did shorten the immediate post-transfusion survival of the labelled erythrocytes. On the other hand, variations in concentrations of chromium from 1.8 to 9.5 micrograms per ml. of whole blood did not have any detectible influence on post-transfusion survival of labelled erythrocytes. Until more data are obtained on the upper limits of chromium concentration not toxic to erythrocytes, it is suggested that future survival studies of chromium-labelled erythrocytes be performed on blood exposed to concentrations of chromium of one microgram or less per ml. of whole blood. The specific activity of radioactive sodium chro-

mate currently available is of sufficient magnitude to allow satisfactory red blood cell survival experiments to be conducted at this level of chromium concentration. Survival of chromated cells over prolonged periods after transfusion appears to be somewhat less than the customarily accepted mean survival time of 120 days. It is possible that further studies may show that these particular observed values may be within the normal biologic range of erythrocyte survival. If this shortening of survival by 10 per cent is found to be constant, however, it should not invalidate the possible value of long-term survival studies in which relatively large changes in rates of erythrocyte destruction are in question (*e.g.*, in acquired hemolytic anemia, or during induced hemolytic states). Preliminary observations indicate that the method yields valuable information in studying the survival of an individual's own red cells within his own body.

Amount of radiation received by the recipient from injected Cr^{51} [11]

If the Cr^{51} which leaves the circulation becomes evenly distributed throughout all the body tissues, the circulating blood would contain the greatest concentration of Cr^{51}. The amount of Cr^{51} giving no more than 0.3 rep during one week would be 320 microcuries for a subject with a blood volume of 5000 ml. (16). Preliminary data obtained in dogs indicate that one week after the transfusion of poorly preserved blood,[12] the Cr^{51} which left the circulation was not evenly distributed throughout all the tissues, but was present in highest concentration in the spleen, liver, and bone marrow. The concentration of Cr^{51} per gram of spleen was 10 times the concentration occurring in the liver and bone marrow so that the spleen contained one-third of the injected dose. If one assumes a similar type of distribution in human subjects, an individual experiencing very rapid hemolysis of donor cells (*i.e.*, 50 per cent or more in one day), which contained 320 microcuries, would be exposed to no more than 1.7 rep per week delivered to the spleen. In this case it would be the dosage received by the spleen which would be the limiting factor in the amount of Cr^{51} which could be safely injected. In experiments where fresh ACD blood containing 320 microcuries is transfused, the spleen of the recipient would receive no more than 0.8 rep per week. The above calculations are conservative inasmuch as it is assumed that all the Cr^{51} going to the spleen remained there and was not cleared from this tissue and that no Cr^{51} was excreted.

Practical application of the radioactive chromate erythrocyte survival method

In the light of our present information, the radioactive chromate method appears ideally suited for evaluation of immediate post-transfusion survival of erythrocytes, either freshly drawn or stored blood. The method is technically simple, highly accurate, can be performed with small aliquots of blood, allows study of the survival of an individual's own erythrocytes in his own vascular system. It makes possible transfusion studies without the hazard of transmitting virus hepatitis, and is harmless from the standpoint of the hazard of radiation.

It is of definite value in following short-term variations in erythrocyte survival and possibly may be proven to be of value in studying small changes in long-term red cell survival.

SUMMARY

1. Erythrocytes in ACD whole blood mixture combine rapidly and firmly with the radioactive chromate ion. After equilibration for three hours at 4 to 6° C., 60 per cent was bound to the red blood cells and 90 per cent was fixed after equilibration for one hour at 25 to 26° C. This is true of both freshly drawn blood and blood stored at 4° C. for as long as thirty-six days. The concentration of this chromate salt shows no marked effect on the rate of Cr^{51} uptake within the limits of 0.25 to 9.5 micrograms. The rate of erythrocyte Cr^{51} uptake increased inversely with the pH within the observed range of 6.0 to 7.2.

2. Post-transfusion survival as determined by the selective agglutination method of erythrocytes tagged with sodium chromate in concentrations of less than 10 micrograms per ml. of whole blood is 2 to 10 per cent shorter than the post-transfusion

[11] The authors wish to express their appreciation to Dr. Gerald J. Hine for valuable aid and criticism in calculation of the radiation doses presented.

[12] 85 per cent of injected cells were destroyed in one week.

survival of nonchromated cells. This somewhat shorter survival of the chromated cells may or may not be within the range of the normal biological variations.

3. $Na_2Cr^{51}O_4$ released from destroyed transfused erythrocytes is not re-utilized to tag the recipient's own red blood cells.

4. The concentration of Cr^{51} in the circulating donor erythrocytes decreases slowly at an exponential rate with a half-life 77 ± 12 days. This may be due to elution of Cr^{51} from the circulating donor cells.

5. The survival of stored blood can be measured accurately by the chromium tagging technique for a period of forty-eight hours after transfusion without correcting for the small amount of chromium leakage. If corrections for the amount of Cr^{51} elution can be made, the method would allow measurement of post-transfused erythrocytes for periods of as long as three months.

REFERENCES

1. Ross, J. F., and Chapin, M. A., Effect of storage of citrated blood on the survival of transfused erythrocytes. J. A. M. A., 1943, **123**, 827.
2. Ross, J. F., Finch, C. A., Peacock, W. C., and Sammons, M. E., The *in vitro* preservation and post-transfusion survival of stored blood. J. Clin. Invest., 1947, **26**, 687.
3. Gibson, J. G., Peacock, W. C., Evans, R. D., Sack, T., and Aub, J. C., The rate of post-transfusion loss of non-viable stored human erythrocytes and the re-utilization of hemoglobin-derived radioactive iron. J. Clin. Invest., 1947, **26**, 739.
4. McKerns, K. W., and Denstedt, O. F., The use of sulfhemoglobin for labeling erythrocytes *in* Preservation of the Formed Elements and of Proteins of the Blood, C. J. Potthoff, ed., Washington, D. C., American National Red Cross, 1949, p. 4.
5. London, I. M., Shemin, D., West, R., and Rittenberg, D., Heme synthesis and red blood cell dynamics in normal humans and in subjects with polycythemia vera, sickle-cell anemia, and pernicious anemia. J. Biol. Chem., 1949, **179**, 463.
6. Moore, C. V., Red cell survival measured with C^{14} tagged globin *in* Preservation of the Formed Elements and of Proteins of the Blood, C. J. Potthoff, ed., Washington, D. C., American National Red Cross, 1949, p. 10.
7. Ross, J. F., A consideration of various techniques of evaluating post-transfusion survival of erythrocytes *in* Conference on Differential Agglutination of Erythrocytes, Washington, D. C., 17 Sept., 1952, Committee on Blood and Related Problems, National Research Council, Division of Medical Science.
8. Ebaugh, F. G., Ross, J. F., and Emerson, C. P., Metabolism of radioactive zinc[65]. To be published.
9. Gray, S. J., and Sterling, K., The tagging of red cells and plasma proteins with radioactive chromium. J. Clin. Invest., 1950, **29**, 1604.
10. Sterling, K., and Gray, S. J., Determination of the circulating red cell volume in man by radioactive chromium. J. Clin. Invest., 1950, **29**, 1614.
11. Lyon, W. S., Disintegration of Cr^{51}. The Physical Rev., 1952, **87**, 1126.
12. Gibson, J. G., Peacock, W. C., Seligman, A. M., and Sack, T., Circulating red cell volume measured simultaneously by the radioactive iron and dye methods. J. Clin. Invest., 1946, **25**, 838.
13. Chapin, M. A., and Ross, J. F., The determination of the true cell volume by dye dilution, by protein dilution, and with radioactive iron. The error of the centrifuge hematocrit. Am. J. Physiol., 1942, **137**, 447.
14. Vazquez, O. N., Newerly, K., Yalow, R. S., and Berson, S. A., Determination of trapped plasma in the centrifuged erythrocyte volume of normal human blood with radioiodinated (I^{131}) human serum albumin and radiosodium (Na^{24}). J. Lab. & Clin. Med., 1952, **39**, 595.
15. Emerson, C. P., The methodology of experimental transfusions and selective agglutination counting in studies on blood preservation, Washington, D. C., 17 Sept., 1952, Committee on Blood and Related Problems, National Research Council, Division of Medical Science.
16. Marinelli, L. D., Quimby, E. H., and Hine, G. J., Dosage determination with radioactive isotopes. II. Practical considerations in therapy and protection. Am. J. Roentgenol., 1948, **59**, 260.

SOME CLINICAL, BIOCHEMICAL AND GENETIC OBSERVATIONS ON HEMOGLOBIN C

By HELEN M. RANNEY, DANIEL L. LARSON,[1] AND GEORGE H. McCORMACK, JR.

(From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital in the City of New York, N. Y.)

(Submitted for publication July 1, 1953; accepted August 3, 1953)

Recent observations (1, 2) in certain American Negroes, of a previously unrecognized abnormal hemoglobin, now designated as hemoglobin C,[2] constitute a significant advance in knowledge of familial anemias. The introduction of filter paper electrophoresis (3, 4) for identification of abnormal hemoglobins (5, 6) has facilitated the study of specimens of hemoglobin from a large number of subjects. With this laboratory procedure, the presence of hemoglobin C alone or in combination with another hemoglobin can readily be detected.

This report deals with the clinical and hematological manifestations which have been encountered in patients with hemoglobin C. Of the total of twelve individuals included in the present study, one patient was found to have hemoglobin C alone, and thus represented the homozygous state for the gene for this abnormal hemoglobin. Five patients had the combination of genes for sickle and C hemoglobin and therefore belong to the group of patients with "sickle cell-hemoglobin C disease" (7) first described by Kaplan, Zuelzer, and Neel (1). The findings in six individuals with a mixture of hemoglobin C and normal hemoglobin are summarized in the discussion. The finding of a patient whose hemoglobin was entirely of the "C" variety afforded an opportunity to study some of the physico-chemical characteristics of this abnormal hemoglobin, which are included in the present report.

[1] Present address: Army Medical Service Graduate School, Washington, D. C.

[2] The designation of hemoglobins in this report conforms with the recommendations of the Symposium of the Hematology Study Section of the Division of Research Grants of the National Institutes of Health (Blood, 1953, **8**, 386).

CASE HISTORIES

Homozygosity for the gene for hemoglobin C: "Hemoglobin C Disease"

M. B., a Negro housewife, 35 years of age, had two brief episodes of joint pains at age 21 and at age 24. Each episode consisted of two to three days of swelling and of tenderness of wrist and interphalangeal joints with prompt and complete clearing of symptoms thereafter. She had four uneventful pregnancies and four living children. The patient was first seen here in 1946 at the age of 29, for arthralgias and urticaria of one week's duration. Physical examination revealed a small, well developed, well nourished young Negro woman who appeared neither acutely nor chronically ill. No unusual features of skeletal development were noted. The spleen was palpable 5 cm. below left costal margin, urticarial lesions were scattered over the face, trunk and extremities, and the right ankle joint was noted to be slightly swollen and tender.

Laboratory studies showed a hemoglobin of 10 Gm. per 100 ml., hematocrit of 31 per cent, red blood cell count of 3.2 million per cu. mm., leucocyte count of 10,400 per cu. mm., with a normal differential count. The stained bloodsmear showed nearly 100 per cent target cells with anisocytosis, poikilocytosis and polychromatophilia. Reticulocytes numbered 3.3 per cent, with platelets of 212,000 per cu. mm. Sickling preparation was negative. An osmotic fragility test showed decreased fragility to hypotonic saline (range 0.375 per cent to 0.20 per cent for patient's erythrocytes; control 0.45 per cent to 0.30 per cent). The sedimentation rate was 6 mm. in one hour (Westergren). The serum bilirubin was 1.2 mg. per cent. Urinalysis was negative and cephalin flocculation test was negative. The serum albumin was 4.8 Gm. per cent and serum globulin was 2.2 Gm. per cent. X-rays of the chest revealed normal heart size. No bony abnormalities were noted in x-rays of the skull or chest.

Urticaria and joint pains subsided within a few days and have not recurred. The patient has been seen frequently during the last six years and has been completely asymptomatic. The splenomegaly has not changed during that time. She received 1.2 Gm. of ferrous sulfate daily for five years; however, the hemoglobin level has remained between 10 and 12 Gm. per 100 ml. with reticulocytes of 3 or 4 per cent. Many target cells have been present on all stained blood smears. Bone marrow aspiration showed erythroid hyperplasia. Coombs' test was negative, repeated sickling tests, employing either

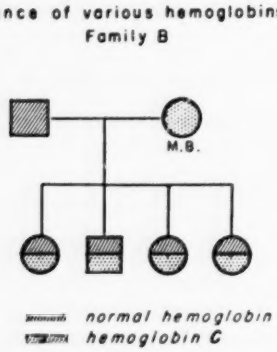


FIG. 1. PATIENT (M. B.) HOMOZYGOUS FOR THE GENE FOR HEMOGLOBIN C

sealed wet preparations or sodium metabisulfite, have been negative and erythrocytes have continued to show decreased osmotic fragility. Mechanical fragility (8) was slightly increased, as shown by values of 9 per cent and 11 per cent (normal less than 6 per cent by method used in this laboratory). In 1952, filter paper electrophoresis showed that all of the hemoglobin of this patient was of the hemoglobin C variety.

The incidence of hemoglobin C in the family of this patient is depicted in Figure 1.

The combination of genes for sickle cell hemoglobin and hemoglobin C: "Sickle cell-hemoglobin C disease"

Case I

M. S., a 15-year-old Puerto Rican boy, was first seen in 1952 because of pain in the right knee of 12 hours' duration. The family history revealed that he had one younger sister who had attacks of joint pains accompanied by jaundice. His maternal great-grandmother was said to have been Negro. Since the age of five years, the patient had had attacks of migratory polyarthritis which were transient and cleared without residual joint deformity. With several of these episodes of arthritis, he was thought to have been icteric.

Physical examination revealed a short, well developed Puerto Rican boy with pain in the right knee. There was no jaundice or cardiac murmur. The liver and spleen were not palpable. The right knee showed slight swelling with tenderness, increased heat and limitation of motion. No other joints were involved.

Laboratory study revealed a hemoglobin of 12 Gm. per 100 ml., erythrocytes of 3.8 million per cu. mm., white blood cells of 5700 per cu. mm. with a normal differential count. Many target cells were noted in the stained smear. Sickle cell preparation was positive. Reticulocytes were 2.9 per cent. Serum bilirubin was 0.9 mg. per cent. An electrocardiogram was within normal limits and the chest x-ray showed questionable cardiac enlargement. Roentgenograms of the skull, chest and knees showed no bony abnormalities. The pain in the right knee subsided within a few days and the patient was asymptomatic thereafter. The filter paper electrophoretic pattern of the hemoglobin of this patient revealed a mixture of sickle cell and C hemoglobin. Subsequently blood specimens of other members of the family were received from Puerto Rico; the distribution of various hemoglobins in members of this family is shown in Figure 2. The sister, who also had recurrent bouts of arthritis and jaundice, had a hemoglobin pattern identical with that of the patient. The patient's family lived in Puerto Rico and was unavailable for other studies.

Case II

C. S., a 22-year-old Negro male, was first seen in 1951 when he had a brief episode of pain in the low back and in the left testicle. Five years before, an episode of arthritis of the hips and knees, followed by painless hematuria, had been called acute rheumatic fever. Physical examination in 1951 was normal except for a spleen palpable 5 cm. below the left costal margin. Laboratory studies revealed a hemoglobin of 10.4 Gm. per 100 ml., erythrocyte count of 3.9 million per cu. mm., leucocytes of 6900 per cu. mm., with a normal differential count. Many target cells were noted on the stained smear. Sickling preparation was positive and ESR was 11 mm. in one hour (Westergren).

In 1951, painless hematuria recurred. Retrograde pyelograms showed changes suggestive of pyelonephritis. Urine specimens were negative for tubercle bacilli. An electrocardiogram was within normal limits; chest x-ray showed slight cardiac enlargement. Except for hematuria, the patient has remained asymptomatic. Filter paper electrophoresis of his hemoglobin in 1952 showed a mixture of sickle and C hemoglobin. Results of further hemoglobin studies carried out on members of this family are depicted in Figure 3.

Case III

C. W., a Negro woman, was first seen in 1948 at the age of 24 during her first pregnancy. She had had re-

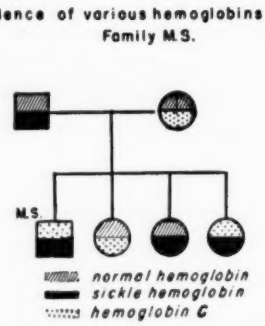


FIG. 2. PATIENT (M. S.) HAS COMBINATION OF GENES FOR HEMOGLOBIN C AND SICKLE CELL HEMOGLOBIN

current bouts of arthralgias, sometimes accompanied by swelling of the joints, during the past 11 years. No abnormalities were noted on physical examination during the third month of pregnancy. Pregnancy proceeded uneventfully until the eighth month when the patient developed pleuritic pain and fever with changes in the chest x-ray compatible with pneumonia or with pulmonary infarctions. With this illness the hemoglobin fell to 4.8 Gm. per 100 ml. (from a level of 9.5 Gm. per 100 ml. during the third month of pregnancy), and the white blood cell count was found to be 3070 per cu. mm. There were 11 per cent myelocytes and 95 nucleated erythrocytes per 100 leucocytes on the stained blood smear. No increase in reticulocytes occurred; osmotic fragility was decreased (0.32 per cent to < 0.28 per cent saline for erythrocytes of patient; control 0.40 per cent to 0.32 per cent saline). Premature labor began on the third day of hospitalization, and a living male infant was delivered. The pulmonary lesions and fever slowly subsided, and the anemia responded satisfactorily to blood transfusions. When the patient was seen one month later, splenomegaly was noted, the hemoglobin had risen to 12 Gm. per 100 ml. and sickling was demonstrated for the first time. Since then, blood smears have repeatedly shown numerous target cells with anisocytosis and polychromatophilia. The patient has been well except for aching pains in the left leg. X-rays of the left hip have shown progressive sclerosis and flattening of the left femoral head, probably the result of ischemic necrosis. Filter paper electrophoretic analysis of her hemoglobin, done in 1952, revealed the combination of sickle cell hemoglobin and hemoglobin C.

Case IV

M. D., a Negro girl was first seen in the Babies' Hospital in 1951 at the age of 12 for low-back pain of six hours' duration. There was no history of any previous bone or joint pain and six hours after its onset the back pain had disappeared. Physical examination was entirely negative except for a palpable spleen tip. Laboratory studies showed hemoglobin to be 6.0 Gm. per 100 ml., leucocytes numbered 10,900 per cu. mm. with 84 per cent polymorphonuclear leucocytes. Reticulocytes were 3.9 per cent and a sickling preparation was positive. She had a low-grade fever during the early part of hospitalization and there was a transient increase in the splenomegaly. Since hospitalization she has been completely asymptomatic and the hemoglobin level has ranged from 8 to 11 Gm. per 100 ml. Her hemoglobin was studied in 1952 and found to be a mixture of sickle hemoglobin and hemoglobin C.

Case V

E. H., a Negro boy, was first admitted in 1952 at the age of 12, for weight loss of two and one-half months' duration. The patient had had an episode of painless hematuria two and one-half months before admission and although the hematuria disappeared, weight loss had continued since that time. There was no previous history of anemia, of bone or joint pain or of jaundice.

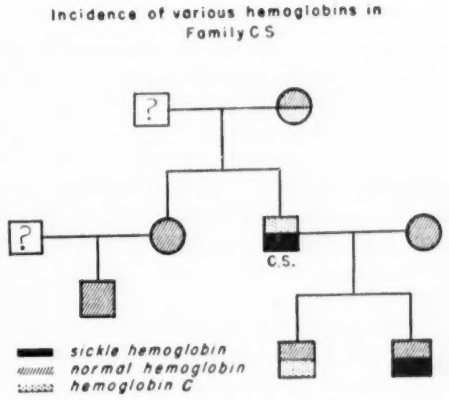


FIG. 3. PATIENT (C. S.) HAS COMBINATION OF GENES FOR HEMOGLOBIN C AND SICKLE CELL HEMOGLOBIN

Physical examination revealed an alert well developed boy not appearing ill. The liver was slightly enlarged and the spleen was felt 4 cm. below left costal margin. Laboratory studies revealed a hemoglobin of 10.5 Gm. per 100 ml. with white blood cells 4600 per cu. mm. and a normal differential count. Numerous target cells were seen on the blood smear. Two sickling preparations were negative. Increased resistance to hypotonic saline was noted in the osmotic fragility test (range from 0.425 per cent to 0.225 per cent saline for patient's erythrocytes with control range from 0.500 per cent to 0.375 per cent saline).

X-rays of the chest showed tuberculosis of the childhood type in the right lung, and gastric aspiration was positive for acid-fast bacilli. Urological studies, including retrograde pyelography and examinations of specimens of urine for tubercle bacilli yielded no evidence of genito-urinary tuberculosis.

Further studies, including G.I. series, barium enema and intravenous urography, were all within normal limits. Because there was some uncertainty about the nature of the left upper quadrant mass, an exploratory celiotomy was done. The mass was identified as an enlarged spleen, and a splenectomy was performed. The histological examination of the spleen suggested sickle cell anemia and subsequently, several sickling preparations were positive. The patient recovered uneventfully from the operation. However, since discharge from the hospital, chest x-rays have shown progression of the pulmonary tuberculosis.

The slight anemia was apparently not affected by splenectomy; one year after the operation the hemoglobin was 10.6 Gm. per 100 ml. with leucocytes of 7000 per cu. mm., and reticulocytes of 2.4 per cent. Filter paper electrophoresis, carried out in 1953, revealed this patient's hemoglobin to be a mixture of sickle hemoglobin and hemoglobin C.

GENETIC STUDIES

The results of hemoglobin studies in members of three families in which hemoglobin C occurred, are shown in Figures 1 to 3. In family M. S., three types of offspring resulted from the mating of a male with sickling trait and a female with hemoglobin C trait. Two of the children had the doubly abnormal heterozygous state for genes responsible for hemoglobin since their hemoglobin was a mixture of sickle and C hemoglobin; a third child had hemoglobin C trait and a fourth had sickling trait. The fourth possible combination, *i.e.*, of entirely normal hemoglobin, occurred in none of the offspring.

Patient M. B. (homozygous for the gene for hemoglobin C), her husband, whose hemoglobin was normal, and their four children provided an unusual opportunity to study the mode of inheritance of hemoglobin C. Each of the four children was found to have the combination of hemoglobin C and normal hemoglobin. That all of these children, genetically first generation (F_1) hybrids, are uniform in their hereditary traits with respect to hemoglobin, is strong evidence that the presence of hemoglobin C is determined by a gene which is transmitted as a simple, although incomplete, Mendelian dominant.

This hypothesis first formulated by Kaplan, Zuelzer, and Neel (1), received further support from the studies in family C. S. In this family, where C. S., the father, had two abnormal hemoglobins (sickle cell and C hemoglobin), and the mother had normal hemoglobin, one son had a mixture of sickle and normal hemoglobin, and the other son, a mixture of hemoglobin C and normal hemoglobin.

Studies of hemoglobin C

The studies of hemoglobin C employed hemoglobin specimens prepared by the method of Drabkin (9) from the blood of patient M. B.

Filter paper electrophoresis was carried out on samples of hemoglobin C, normal hemoglobin and sickle hemoglobin; dye elution curves, prepared by the method previously described (6), are shown in Figure 4. Under the conditions employed for filter paper electrophoresis, hemoglobin C has the lowest mobility of any hemoglobin studied. The great difference in mobility of hemoglobin C and the other hemoglobins makes filter paper electrophoresis a suitable technique for the identification of this abnormal hemoglobin.

Moving boundary electrophoresis was carried out on specimens of hemoglobin C. Veronal

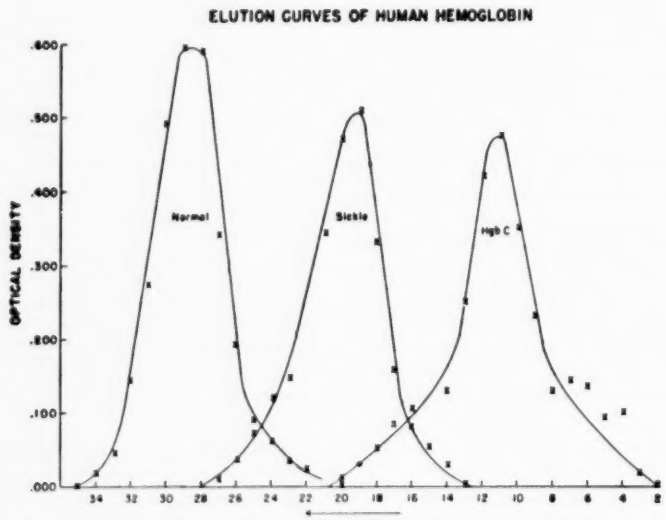


FIG. 4. FILTER PAPER ELECTROPHORESIS—DYE ELUTION TECHNIQUE
Abscissa represents distance in centimeters from original site of placement of hemoglobin specimens (6).

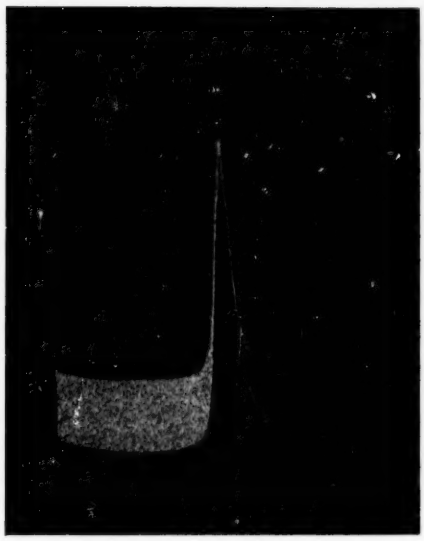


FIG. 5. ASCENDING BOUNDARY OF HEMOGLOBIN C IN TISELIUS APPARATUS

Conditions of analysis are given in text.

buffer, ionic strength 0.1, pH 8.6 was employed for these analyses; a preliminary 24-hour dialysis of the hemoglobin specimens against the veronal buffer was carried out. Values for mobility were the same for ascending and descending boundaries: 2.6×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$.

A photograph of the ascending boundary of one of these specimens is shown in Figure 5.

That there is a small amount of skewing present in this pattern is obvious. This may be the result of impurities in the hemoglobin specimen which was not crystallized, or it may result from an admixture with a small amount of another type of hemoglobin.

The electrophoretic properties of human hemoglobins including mobility studies have been summarized by Itano (7) who found the mobility of hemoglobin C in cacodylate buffer at pH 6.5 to be 3.2×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$.

The ultra-violet absorption spectrum of hemoglobin was determined with a Beckman DU spectrophotometer. The absorption spectrum of hemoglobin C was found to be the same as that of normal hemoglobin.

Ultra-centrifugation studies of hemoglobin C yielded a sedimentation constant of 4.4×10^{-13}. This is in good agreement with values of $4.4_8 \times 10^{-13}$ obtained for normal hemoglobin by Pedersen (10).

Alkali denaturation of a sample of hemoglobin C by the method of Singer, Chernoff, and Singer (11), showed 0.7 per cent alkali resistant hemoglobin. This is within the range of values obtained for normal hemoglobin.

The characteristic electrophoretic mobility, demonstrable by either moving boundary or filter paper electrophoresis, provides the only laboratory method for the identification of hemoglobin C at this time. Studies of the immunological properties and of the crystalline character of hemoglobin C are in progress.

DISCUSSION

Clinical and Hematological Manifestations Resulting from the Presence of Hemoglobin C

The homozygous state with respect to the gene for hemoglobin C

Since only one patient (M. B.), whose hemoglobin consists entirely of hemoglobin C was studied, no general conclusions can be drawn about the clinical syndrome. However, certain clinical and hematological features of the present case will be pointed out. This patient had virtually no symptoms except for three episodes of transient arthritis. She had splenomegaly for many years and the hemoglobin level remained at 10 to 12 Gm. per 100 ml. with mild reticulocytosis and erythroid hyperplasia of bone marrow. The stained smears showed nearly all the erythrocytes to be target cells. In contrast with many patients with sickle cell disease, this patient with "hemoglobin C disease" never had crises with anemia and had four uneventful pregnancies.

Although this patient had few manifestations of her hematologic disorder, her erythrocytes exhibited more marked abnormalities on stained blood smear (greater number of target cells and more marked poikilocytosis) than did the erythrocytes of any of the other patients in this report.

Kaplan, Zuelzer, and Neel (1) and Neel, Itano, and Lawrence (12) suggested that the homozygous state for the gene responsible for hemoglobin C might correspond to a previously recognized hematologic entity. Some of the reported cases of Mediterranean anemia occurring in Negroes may

well have been individuals homozygous for hemoglobin C. In four such cases reported by Schwartz and Mason (13) large numbers of target cells were found, in addition to splenomegaly and decreased erythrocyte fragility. However, in two of their cases hemoglobin values were approximately 50 per cent, and in all, erythrocyte counts were disproportionately elevated with respect to the corresponding hemoglobin levels. It is, therefore, not possible to conclude from the available data whether any reported cases of Mediterranean anemia in the Negro were in reality examples of the homozygous state for hemoglobin C. It seems preferable to classify M. B., the case reported here, as an example of "hemoglobin C disease," rather than to attempt to clarify the relationship to previously reported patients in whom electrophoretic studies of the hemoglobin were not done.

The combination of the genes for sickle cell hemoglobin and hemoglobin C

The outstanding clinical and hematological features of patients with the combination of the genes for sickle and C hemoglobin are shown in Table I. This syndrome has been designated "sickle cell-hemoglobin C disease" (7). Some clinical features which aid in distinguishing this disease state from sickle cell anemia have been pointed out by Kaplan, Zuelzer, and Neel (1). The course is more benign, the anemia milder, and there is progressive splenic enlargement. It should be noted that the patients of the present study were somewhat older than those reported by Kaplan. In our patients, the anemia was milder and symptoms were fewer than in most patients with sickle cell anemia. However, one patient (M. S.) did not have a palpable spleen and another (M. D.) has a barely palpable spleen tip. In none of the patients has there been significant progression of the splenomegaly during the period of observation. Splenectomy, which was performed in one patient, did not correct the mild anemia.

An outstanding feature in the history of three of the patients was the occurrence of arthritis. In four patients (including the sister of M. S.) arthritis had been a major complaint. While polyarthritis occurs frequently in sickle cell anemia, the fact that it occurred in four of these patients with the combination of sickle and C hemoglobin as well as in patient M. B. who had only hemoglobin C, is of interest. Obviously too few cases have been studied to draw any conclusions at this time.

Bone changes, which are frequently seen in classical sickle cell anemia were found in only two patients. One, patient E. H., had cortical defects in

TABLE I

Summary of clinical and hematological findings in five patients with the combination of the genes for sickle cell hemoglobin and hemoglobin C

Patient	Present age	Joint symptoms	Anemic crises	Splenomegaly*	X-ray changes in bones	Usual hemoglobin levels Gm. %	Reticulocytes %	Target cells %
M. S.	15	frequent arthritis	†	none	none	11–12	3	many (20–40%)
C. S.	24	one severe attack of arthritis	†	5 cm.	none	10–13	4	many (30–40%)
C. W.	27	frequent arthralgias	yes	3 cm.	ischemic necrosis left femoral head	10–12	3–6	many (30–40%)
M. D.	13	one attack back pain	yes	barely palpable	none	8–11	4	occasional (4%)
E. H.	13	none	†	4 cm.	cortical defects long bones	10–12	1–2	frequent (10%)

* Figure refers to centimeters palpated below left costal margin.
† No anemic crises ever documented.

several long bones, which are of uncertain significance. The other patient (C. W.), however, has a severe deformity of the left femoral head, roentgengraphically indistinguishable from bony lesions associated with classical sickle cell anemia.

The hematological findings in these patients were similar to those previously reported (1). Nearly normal hemoglobin values, with a mild reticulocytosis, were found in all the patients. Two patients had crises accompanied by more severe anemia, but higher hemoglobin levels were quickly regained. Target cells were found on stained blood smears of all of the patients; in three of the patients, target cells constituted 50 to 60 per cent of the erythrocytes, but in two patients, target cells were as few as 10 and 4 per cent.

From the findings in the few recognized cases of the syndrome which accompanies the combination of sickle and hemoglobin C, it appears that this disorder has manifestations as variable as those of classical sickle cell anemia. At the present time it is not possible to arrive at the diagnosis of hemoglobin C in combination with sickle hemoglobin on clinical grounds alone. In adult patients, the clinical picture of sickle cell anemia may be extremely variable, and patients have been encountered who had some or all of the clinical and hematological features ascribed to the combination of sickle and C hemoglobin, but whose hemoglobin was entirely of the sickle variety as determined by filter paper electrophoresis.

The combination of the genes for hemoglobin C and normal hemoglobin

Six subjects with hemoglobin C trait were studied; they were completely symptom free and in none were any abnormalities found on physical examination. The hemoglobin values ranged from 11 to 12 Gm. per 100 ml. (within normal limits for the age groups to which the patients belonged). Reticulocytes were not significantly elevated, values ranging from 0.6 to 1.6 per cent. The occurrence of target cells in association with hemoglobin C trait has been noted previously (1); a few target cells were seen in the blood smears of all the individuals with hemoglobin C trait encountered in this study. In addition, mild degrees of hypochromia were noted in a few blood smears, but neither the number of target cells nor the degree of hypochromia was striking.

SUMMARY

The clinical and hematological findings in a patient whose hemoglobin was entirely of the hemoglobin C type have been described. The outstanding features were splenomegaly, striking numbers of target cells on stained blood smears, mild reticulocytosis, and a nearly normal hemoglobin level.

Five patients whose hemoglobin was a mixture of sickle and C hemoglobin were described. Although the clinical picture may be variable, most of these patients had splenomegaly with nearly normal hemoglobin values and a mild reticulocytosis. Two of the patients had crises with anemia and one patient had roentgenographic evidence of bone infarction.

Studies of the occurrence of hemoglobin C in various families confirm the theory proposed by Kaplan, Zuelzer, and Neel (1) that the presence of hemoglobin C is determined by a gene which is transmitted as a simple Mendelian dominant.

Studies of hemoglobin C including electrophoresis, ultra-centrifugation, ultra-violet absorption spectrum and alkali denaturation indicate that this abnormal hemoglobin can be identified only by electrophoresis at this time.

ADDENDA

Since this paper was submitted for publication, a patient homozygous for hemoglobin C has been described by T. Spaet (Pediatrics, In press).

Also, we have since encountered seven additional patients with the combination of the genes for sickle and C hemoglobin. Features of sickle cell hemoglobin C disease in these additional patients are similar to those reported.

ACKNOWLEDGMENT

The authors are grateful to Dr. James A. Wolff, of the Babies' Hospital, New York City, for permission to publish Cases 4 and 5; and to Grace Vanderhoff, B.S. for valuable technical assistance.

REFERENCES

1. Kaplan, E., Zuelzer, W. W., and Neel, J. V., A new inherited abnormality of hemoglobin and its in-

teraction with sickle cell hemoglobin. Blood, 1951, **6**, 1240.

2. Itano, H. A., and Neel, J. V., A new inherited abnormality of human hemoglobin. Proc. Nat. Acad. Sc., 1950, **36**, 613.
3. Durrum, E. L., A microelectrophoretic and microionophoretic technique. J. Am. Chem. Soc., 1950, **72**, 2943.
4. Kunkel, H. G., and Tiselius, A., Electrophoresis of proteins on filter paper. J. Gen. Physiol., 1951, **35**, 89.
5. Spaet, T. H., Identification of abnormal hemoglobins by means of paper electrophoresis. J. Lab. & Clin. Med., 1953, **41**, 161.
6. Larson, D. L., and Ranney, H. M., Filter paper electrophoresis of human hemoglobin. J. Clin. Invest., 1953, **32**, 1070.
7. Itano, H. A., Human hemoglobin. Science, 1953, **117**, 89.
8. Shen, S. C., Castle, W. B., and Fleming, E. M., Experimental and clinical observations on increased mechanical fragility of erythrocytes. Science, 1944, n.s., **100**, 387.
9. Drabkin, D. L., Spectrophotometric studies. XIV. The crystallographic and optical properties of the hemoglobin of man in comparison with those of other species. J. Biol. Chem., 1946, **164**, 703.
10. Pedersen, K. O., The protein molecule *in* The Ultracentrifuge, by Svedberg, T., and Pedersen, K. O., Oxford, at the Clarendon Press, 1940, Table 48, opp. p. 406.
11. Singer, K., Chernoff, A. I., and Singer, L., Studies on abnormal hemoglobins. I. Their demonstration in sickle cell anemia and other hematologic disorders by means of alkali denaturation. Blood, 1951, **6**, 413.
12. Neel, J. V., Itano, H. A., and Lawrence, J. S., Two cases of sickle cell disease presumably due to the combination of the genes for thalassemia and sickle cell hemoglobin. Blood, 1953, **8**, 434.
13. Schwartz, S. O., and Mason, J., Mediterranean anemia in the Negro. A report of four cases and their families. Blood, 1949, **4**, 706.

THE HEMODYNAMIC EFFECTS OF HYPOTENSIVE DRUGS IN MAN. III. HEXAMETHONIUM [1, 2, 3]

By EDWARD D. FREIS, JOHN C. ROSE, EDWARD A. PARTENOPE, THOMAS F. HIGGINS, ROBERT T. KELLEY,[4] HAROLD W. SCHNAPER, AND ROBERT L. JOHNSON

(From the Cardiovascular Research Laboratory, Georgetown University Hospital, the Department of Medicine, Georgetown University School of Medicine, and the Veterans Administration Hospital, Washington, D. C.)

(Submitted for publication July 7, 1952; accepted August 14, 1953)

While searching for derivatives of d-tubocurarine, Barlow and Ing (1) and Paton and Zaimis (2) independently synthesized a series of polymethylene bistrimethylammonium salts. The latter investigators demonstrated that the pharmacological properties of this series were related to the length of the polymethylene chain; the deca-compound produced neuromuscular block while the penta and hexa-compounds prevented the transmission of impulses across the synapses in all autonomic ganglia. They also carried out preliminary trials in man (3) but Arnold and Rosenheim were the first to use these agents in hypertension (4). Finally, Restall and Smirk demonstrated that it was possible to reduce blood pressure and obtain clinical improvement in hypertensive patients for long periods by the use of repeated parenteral doses of hexamethonium (5).

Studies in this laboratory have confirmed the observations of Restall and Smirk and in addition have shown the potentiating effect of l-hydrazinophthalazine (Apresoline) when alternated with doses of hexamethonium in patients with hypertension (6, 7). Our studies also suggested that hexamethonium may be useful in the treatment of acute peripheral vascular disorders associated with neurogenic vasospasm as well as in the evaluation of the sympathetic vasoconstrictor component in cases of peripheral vascular disease (8). Since from these previous studies it appeared that hexamethonium was a potent agent both for reducing blood pressure and increasing foot and digital blood flow a more complete analysis of its hemodynamic effects seemed indicated.

METHODS

The subjects were 25 hypertensive patients admitted to the wards of Georgetown University Hospital and the Veterans Administration Hospital and four normotensive young males (medical students). Hexamethonium was administered intravenously at a rate of 1 to 2 mg. per minute for the first 15 mg. and then at a rate of 5 mg. per minute, in all instances until a significant hypotensive effect had been obtained or until 50 to 100 mg. had been administered. All dosages refer to the amount of hexamethonium ion administered.

The methods used in this investigation were essentially similar to those described in a previous communication (9), with the following exceptions: the determination of arteriovenous oxygen difference were carried out using the spectrophotometric method of Hickam and Frayser (10). The oxygen content of the expired air was determined using a Pauling type oxygen analyzer.[5] The total peripheral resistance was calculated according to the method of Green, Lewis, Nickerson, and Heller (11). In the muscle blood flow experiments it was necessary to estimate the mean arterial pressure from the values for systolic and diastolic pressure as determined by the auscultatory method. The formula used was as follows: mean arterial pressure = .436 (pulse pressure) + diastolic pressure (12). Calf blood flow was measured as previously described except that a strain gage,[6] with a car-

[1] Supported in part by research grants from the National Heart Institute, U. S. Public Health Service, the Squibb Institute for Medical Research, New Brunswick, New Jersey, Irwin, Neisler and Company, Decatur, Illinois and Ciba Pharmaceuticals, Summit, New Jersey.

[2] Sponsored (in part) by the VA and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[3] Hexamethonium dibromide (Bistrium) was supplied by H. Sidney Newcomer, M.D., E. R. Squibb and Sons, New York, New York.

[4] Research Fellow—Washington, D. C., Heart Association.

[5] Model C, Arnold O. Beckman, Inc., South Pasadena, California.

[6] Model P-23-B, Statham Laboratories, Beverly Hills, California.

rier-wave type amplifier and direct writing oscillograph,[7] replaced the Brodie's bellows apparatus. Glomerular filtration rate was determined by the clearance of sodium thiosulfate (13).

RESULTS

I. Cardiac function

Cardiac output, mean arterial pressure and total peripheral resistance

The changes in cardiac output and total peripheral resistance seemed to vary with the state of cardiac compensation. In the hypertensive patient with a compensated heart the usual response to hypotensive doses of hexamethonium was a slight decrease in cardiac output. Thus, in 11 post-treatment analyses carried out in six patients without heart failure the cardiac output decreased by 6 to 38 per cent (mean 22.0 per cent, S.D. 9.3) (Table I, Cases 1 through 6). The decrease in mean arterial pressure was of similar magnitude varying from 10 to 37 per cent (mean 23.5 per cent, S.D. 7.0). In only one instance did the arterial pressure fall to normotensive levels. The total peripheral resistance did not change significantly varying between + 19 and − 22 per cent (mean − 1.3 per cent).

In contrast, in four additional patients with congestive heart failure and one with malignant hypertension there was a significant increase in cardiac output in three and a slight increase in two patients following hexamethonium. The range for nine post-treatment determination was + 3 to 100 per cent and the mean 38 per cent, S.D. 35.4 (Table I, Cases 7 through 11). In these instances the total peripheral resistance fell significantly the range being 27 to 70 per cent and the mean 47 per cent, S.D. 14.1.

Other aspects of cardiac function

The heart rate usually increased moderately after hexamethonium. There were insignificant changes in rate in three patients, slowing in one and rises in six cases; the average increase was 14 beats per minute (Table I).

Pressures on the right side of the circulation were measured in seven patients and decreased in all instances. In the group with compensated hearts the pulmonary arterial pressure decreased in patient W. C. from 26/14 to 14/8 mm. Hg. In patient B. J. the right ventricular pressure decreased from 38/12 to 22/4 mm. Hg (Figure 1). In subject C. A., the mean right auricular pressure decreased from − 0.5 to − 2.5 mm. Hg. In the decompensated patients the pulmonary arterial pressure decreased in patient T. P. from 45/22 to 20/12 mm. Hg and in subject R. S. from 70/40 to 40/15 mm. Hg. The right ventricular pressures fell in subject J. C. from 115/25 to 85/18 mm. Hg and in subject A. C. from 130/20 to 80/8 mm. Hg. The decline in right heart pressures paralleled the fall in systemic arterial pressure.

II. Blood Flow Through Various Regions

Blood flow through the muscles (calf blood flow)

In a previous communication it was pointed out that a ten-fold increase in foot (primarily skin) blood flow occurred after hexamethonium (14). In the calf segment (primarily muscle) an increased blood flow as determined by the plethysmographic method also was observed but to a much smaller degree than that observed in the foot (Table II).

Ten hypertensive patients were studied. Two patients in the malignant phase had previous therapy with hexamethonium while the remaining eight were patients with essential hypertension who had received no previous treatment with hexamethonium. In the latter eight cases all exhibited an increase of muscle blood flow varying from 11 to 61 per cent (mean 39.4 per cent) of the control values. The crude peripheral resistance decreased by 26 to 56 per cent (mean 40.4 per cent). The increase of blood flow began simultaneously with the decrease of blood pressure and some increase persisted for at least an hour.

The two patients who had malignant hypertension and who had been under continuous dosages of hexamethonium immediately prior to testing showed a decrease of blood flow in one instance and no change in the other. It could not be determined from this limited data whether these atypical responses were associated with the development of tolerance to the vasodilating properties of the drug or represented a response peculiar to patients in the malignant phase of hypertension.

[7] Model 140-C, Sanborn Company, Cambridge, Massachusetts.

TABLE I

Effects of hexamethonium on mean arterial pressure, cardiac rate, cardiac output and total peripheral resistance

Patient and Diagnosis	Sex	Age	Surface area	Control					Hexamethonium I.V.	Time after drug	After hexamethonium				
				Arterial pressure	Mean arterial pressure	Cardiac rate	Cardiac output	Total peripheral resistance			Arterial pressure	Mean arterial pressure	Cardiac rate	Cardiac output	Total peripheral resistance
			sq. cm.	*mm. Hg*	*mm. Hg*	*per min.*	*L. per min.*	*units**	*mg.*	*minutes*	*mm. Hg*	*mm. Hg*	*per min.*	*L. per min.*	*units**
M. C. Essential hypertension	M	63	1.75	220/115 220/115	148 148	80 80	6.4 6.3	.023 .024	10	7 18	140/95 140/95	111 110	84 80	5.5 5.2	.020 .021
A. F. Essential hypertension	M	31	1.96	250/140 250/140	175 175	81 79	5.4 5.0	.033 .035	15	10 23	210/110 215/120	140 158	94 90	3.9 4.6	.036 .034
J. J. Essential hypertension	M	59	1.78	215/120 215/120	152 152	83 71	6.3 6.1	.024 .025	25	8	130/100	112	75	5.8	.019
C. A. Essential hypertension	M	58	1.98	220/120 225/120	152 154	93 90	7.1 7.4	.021 .021	37	6 19	145/100 125/90	114 106	103 98	4.5 5.2	.025 .020
B. J. Essential hypertension	M	26	1.73	185/115 180/120	152 156	85 82	6.6 6.7	.023 .023	30	9 18	148/100 145/95	116 113	100 106	4.5 5.3	.026 .022
W. C. Essential hypertension	M	34	1.78	240/130 240/130	164 164	90 90	6.6 6.3	.025 .026	50	14 24	180/110 178/110	136 134	103 103	5.0 5.7	.027 .024

* $\frac{\text{Mean femoral arterial pressure in mm. Hg}}{\text{Cardiac output in ml. per minute}}$.

TABLE I—*Continued*

Patient and Diagnosis	Sex	Age	Surface area	Control					Hexamethonium I.V.	Time after drug	After hexamethonium				
				Arterial pressure	Mean arterial pressure	Cardiac rate	Cardiac output	Total peripheral resistance			Arterial pressure	Mean arterial pressure	Cardiac rate	Cardiac output	Total peripheral resistance
			sq. cm.	*mm. Hg*	*mm. Hg*	*per min.*	*L. per min.*	*units**	*mg.*	*minutes*	*mm. Hg*	*mm. Hg*	*per min.*	*L. per min.*	*units**
T. P. Essential hypertension with heart failure. (Digitalized)	M	68	1.86	265/140 275/145	194 202	82 84	4.8 4.5	.040 .045	7	8 13	200/115 235/120	150 156	90 90	5.0 4.8	.030 .031
J. C. Essential hypertension with heart failure	M	53	1.70	200/110 200/110	145 145	91 90	4.6 4.3	.030 .034	8	8	120/90	105	91	6.1	.017
A. C. Essential hypertension with heart failure	F	49	2.01	260/150 220/150	188 182	90 85	5.8 6.4	.033 .028	18	7 13	152/84 140/80	114 106	92 75	7.9 6.4	.014 .017
R. S. Essential hypertension with heart failure	M	52	1.80	190/105 188/106	130 130	104 100	2.6 1.9	.050 .068	24	12 22	100/64 114/76	80 90	85 88	4.5 3.8	.018 .024
T. N. Malignant hypertension†	M	32	1.81	215/125 220/125	150 155	107 105	3.5 3.5	.044 .044	30	7 16	155/105 150/100	118 118	135 122	6.0 4.3	.020 .028

† Papilledema.

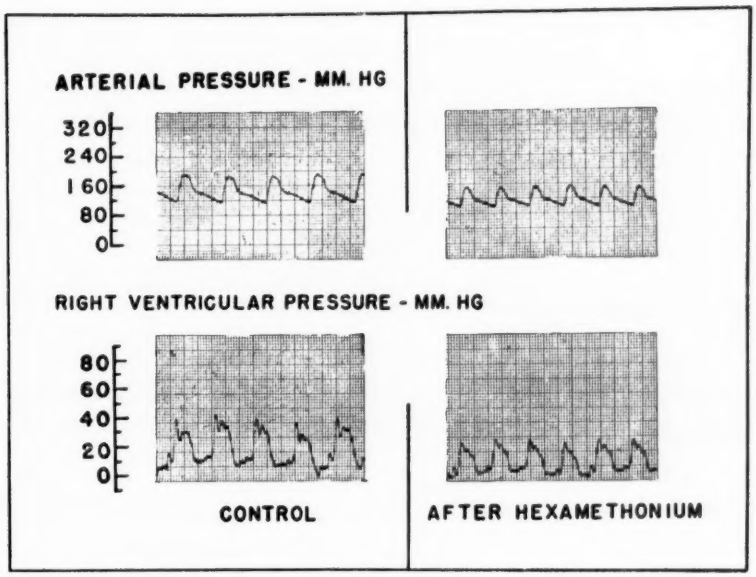


FIG. 1. CUTTINGS TAKEN FROM THE RECORDINGS OF SYSTEMIC ARTERIAL (ABOVE) AND RIGHT VENTRICULAR PRESSURES (BELOW) IN PATIENT B. J. BEFORE AND 19 MINUTES AFTER THE INTRAVENOUS ADMINISTRATION OF 30 MG. OF HEXAMETHONIUM

The reduction of arterial pressure was accompanied by a considerable decrease of right ventricular pressure. See text and Table I for further details.

Blood flow through the hepatic-portal circuit

Determinations of estimated hepatic-portal blood flow were carried out in five hypertensive and two normal subjects. In all of the hypertensive patients the mean arterial pressure decreased following hexamethonium, the range being 20 to 40 per cent and the mean 28 per cent (Table III). In four cases estimated hepatic blood flow decreased by 8 to 54 per cent (mean 25 per cent) while in the remaining case it was unchanged. The hepatic-portal vascular resistance fell in all except one of the hypertensive patients.

One of the normal subjects exhibited insignificant changes in estimated hepatic portal flow and peripheral vascular resistance while in the remaining subject hepatic blood flow decreased 19 per cent and peripheral resistance also was reduced slightly. Thus in four or five hypertensive and one of two normotensive subjects the estimated hepatic portal blood flow decreased moderately and in the remaining two cases did not change significantly.

Blood flow through the kidneys

Two of the seven cases studied exhibited no significant change in renal blood flow (D. G. and H. B., Table IV). However, changes in arterial pressure also were insignificant in these two subjects despite relatively high doses of hexamethonium. In the remaining five cases there was a reduction of renal plasma flow which paralleled the fall in arterial pressure (Table IV and Figure 2). However, in four of these cases all of whom were hypertensives, the renal plasma flow returned to or above the control levels after periods varying from 15 to 50 minutes following the time of drug administration. In all four instances the plasma flow rose despite a continued significant reduction of arterial pressure. In the remaining case, a normal subject, the plasma flow remained reduced up to 50 minutes after hexamethonium. Thus, the usual pattern of response to hypotensive doses of hexamethonium was an early decrease in renal plasma flow followed by a return to control values despite continued reduction of arterial pressure.

TABLE II

Effects of hexamethonium on blood flow in the calf

Patient and Diagnosis	Sex	Age	Control			Hexamethonium intravenously	After hexamethonium					
			Arterial pressure	Blood flow per 100 ml. leg volume	Peripheral resistance		Time after drugs	Arterial pressure	Blood flow per 100 ml. leg volume	Mean change	Peripheral resistance	Mean change
			mm. Hg	*ml. per min.*	*units**	*mg.*	*minutes*	*mm. Hg*	*ml. per min.*	*per cent*	*units**	*per cent*
W. F.	M	50	178/130	5.4	28	30	3	125/95	8.3		13	
Essential			168/125	5.6	26		6	130/92	8.3		13	
hypertension			180/128	5.4	28		10	135/100	8.0		14	
			180/128	4.8	32		20	135/98	8.3		14	
			175/125	5.6	26		35	125/90	8.9		12	
							45	125/90	8.2		13	
							60	125/90	8.2		13	
										+55		−54
J. J.	M	46	165/110	5.0	27	50	4	140/100	6.5		18	
Essential			160/105	4.8	27		8	135/100	7.6		15	
hypertension			160/105	4.1	32		15	135/100	7.1		16	
			180/110	4.7	30		25	140/105	7.7		16	
							35	145/110	7.8		16	
							40	140/110	8.3		14	
										+61		−45
B. R.	M	31	165/115	8.0	17	100	5	155/115	11.9		11	
Essential			170/120	10.1	14		8	165/115	11.7		12	
hypertension			165/120	8.5	17		15	155/110	12.0		11	
			165/115	7.7	18		25	155/110	12.0		11	
										+40		−31
T. N.	M	34	220/140	8.9	20	100	3	185/145	6.5		25	
Essential			220/145	8.2	22		5	178/135	5.8		27	
hypertension.			230/145	6.4	29		6	180/135	5.4		27	
(Formerly							9	175/130	5.1		29	
malignant)							16	165/130	4.8		30	
Hexamethonium							20	170/135	4.1		37	
treated.							28	175/135	4.1		37	
							40	170/135	3.9		38	
							45	170/130	3.8		43	
							55	170/130	3.8		39	
										−40		+42
E. T.	M	36	145/110	3.5	36	14	5	120/90	8.6		12	
Essential			155/110	4.2	31		10	120/90	8.6		12	
hypertension			155/110	2.5	52		20	125/90	5.8		18	
							38	130/90	6.1		18	
							58	130/90	5.3		20	
							63	135/95	5.5		20	
							75	140/95	5.4		21	
										+47		−56

* $\frac{\text{"Mean" arterial pressure (12) in mm. Hg}}{\text{Blood flow in ml. per 100 ml. leg volume per min.}}$

TABLE II—*Continued*

Patient and Diagnosis	Sex	Age	Control			Hexamethonium intravenously	After hexamethonium					
			Arterial pressure	Blood flow per 100 ml. leg volume	Peripheral resistance		Time after drugs	Arterial pressure	Blood flow per 100 ml. leg volume	Mean change	Peripheral resistance	Mean change
			mm. Hg	*ml. per min.*	*units**	*mg.*	*minutes*	*mm. Hg*	*ml. per min.*	*per cent*	*units**	*per cent*
I. E.	M	50	220/140	4.5	39	100	5	220/140	7.0		25	
Malignant			220/140	5.1	34		20	210/140	5.4		32	
hypertension.							35	210/145	4.2		41	
Hexamethonium							50	210/145	4.6		38	
treated										+ 1		− 1
G. H.	M	29	170/110	8.7	16	50	5	140/90	11.6		10	
Chronic			170/110	10.2	13		20	140/90	13.0		9	
glomerulo-			170/110	10.6	13		35	130/85	11.6		9	
nephritis							55	130/85	13.0		8	
							70	145/95	11.5		10	
										+22		−34
B. M.	M	55	175/110	2.0	69	28	3	130/90	2.2		49	
Essential			165/110	1.7	79		4	115/88	2.1		47	
hypertension			175/110	1.8	77		7	120/90	1.8		57	
							9	115/85	1.8		54	
							18	115/85	2.0		49	
							26	130/95	2.3		48	
							37	142/102	2.1		57	
							39	132/95	2.0		55	
										+11		−31
W. A.	M	42	175/125	4.1	36	50	2	148/105	6.7		19	
Essential			172/130	4.3	35		6	145/105	6.7		18	
hypertension			175/125	4.2	35		8	140/105	7.0		17	
			175/125	4.8	31		16	145/105	7.3		17	
			175/125	4.1	36		29	138/110	7.1		17	
							40	125/100	6.0		20	
							50	130/100	5.7		20	
							62	135/105	5.9		20	
										+52		−46
R. C.	M	32	196/130	4.0	40	50	2	164/128	4.6		31	
Essential			196/130	3.7	43		3	160/128	4.9		29	
hypertension			192/130	3.8	41		5	164/125	4.8		30	
			196/130	3.5	45		7	160/130	5.0		35	
							12	170/130	4.6		32	
							24	162/132	4.6		32	
							34	168/134	4.6		32	
							54	166/134	4.9		30	
										+27		−26

TABLE III

Effects of hexamethonium on estimated hepatic blood flow

Patient and Diagnosis	Sex	Age	Surface area	Control			Hexamethonium I.V.	After hexamethonium			
				Mean arterial pressure	E.H.B.F.	Estimated hepatic vascular resistance		Time after drug	Mean arterial pressure	E.H.B.F.	Estimated hepatic vascular resistance
			sq. m.	*mm. Hg*	*ml. per min.*	*units**		*min.*	*mm. Hg*	*ml. per min.*	*units**
C. P.	M	48	1.85	216	592	.365	2	2	118	425	.278
Malignant				184	529	.348		8	108	323	.334
hypertension								14	128	474	.270
								22	124	489	.254
E. G.	M	38	1.90	118	2008	.057	50	11	87	933	.093
Essential				118	1875	.063		24	92	894	.103
hypertension								37	94	932	.101
S. J.	M	52	1.75	165	872	.189	5	7	110	770	.143
Essential				155	924	.168		12	110	776	.142
hypertension								17	110	742	.148
H. C.	M	40	1.97	150	874	.172	10	5	125	793	.158
Essential				160	696	.230		10	130	817	.159
hypertension				140	585	.239					
							18	16	125	524	.236
								24	100	833	.120
E. T.	M	36	1.80	140	1113	.126	15	1	110	1097	.100
Essential				141	1030	.137		5	98	1007	.092
hypertension				144	1089	.132		10	95	883	.108
R. B.	M	29	1.72	70	1792	.039	50	6	63	2155	.029
Normal				70	1496	.047		18	63	1933	.033
				63	1974	.032		29	65	1428	.046
								40	65	1244	.052
P. S.	M	24	2.14	82	1524	.054	100	4	60	1510	.040
Normal				79	1520	.052		12	52	1396	.037
				79	1524	.052		23	58	981	.059
								34	60	1388	.043
								50	66	1416	.047

* $\frac{\text{Mean femoral arterial pressure in mm. Hg}}{\text{Blood flow in ml. per minute}}$.

TABLE IV

Effects of hexamethonium on renal clearances

Patient and Diagnosis	Sex	Age	Surface area	Control					Hexamethonium I.V.	After hexamethonium					
				Arterial pressure	Renal plasma flow	Glomerular filtration rate	Filtration fraction	Urine volume		Time after drug	Arterial pressure	Renal plasma flow	Glomerular filtration rate	Filtration fraction	Urine volume
			sq. m.	*mm. Hg*	*ml. per min.*	*ml. per min.*	*per cent*	*ml. per min.*	*mg.*	*minutes*	*mm. Hg*	*ml. per min.*	*ml. per min.*	*per cent*	*ml. per min.*
A. Z. Essential hypertension	M	52	1.78	248/142	248	69	28	7.9	40	5	218/130	246	72	29	6.2
				248/140	258	69	27	7.5		15	204/130	229	56	24	3.9
										30	198/126	242	59	24	4.2
										50	200/122	272	65	24	4.2
C. S. Essential hypertension	M	38	1.86	172/110	411	88	21	2.6	30	5	85/58	289	73	25	2.5
				166/110	393	93	24	3.1		15	78/58	287	69	24	4.0
										30	89/65	455	62	14	4.6
A. S. Malignant hypertension	M	60	2.26	188/118	366	106	29	6.8	10	10	148/ 92	149	43	29	1.3
				186/114	327	94	29	5.1		30	156/104	373	72	19	1.5
				190/118	418	117	28	5.8		50	168/110	341	79	23	1.5
D. G. Malignant hypertension and uremia	M	28	1.65	208/112	215			1.4	50	10	212/126	234			1.5
				214/118	214			1.5		30	200/120	222			1.4
M. Y. Essential hypertension	F	48	1.89	230/134	448	88	20	7.9	25	5	180/110	296	54	18	3.6
				225/135	368	84	23	7.5		15	176/114	381	70	18	3.7
										30	168/110	343	64	19	3.0
										50	174/110	321	70	22	2.7
H. B. Normal	M	36	1.78	108/76	520	103	20	6.5	80	5	100/70	379	85	23	7.5
				104/72	319	86	27	6.4		15	102/74	581	86	15	7.7
										30	100/74	414	91	22	7.6
										50	104/76	360	74	21	5.0
R. B. Normal	M	29	1.70	100/70	812	203	13	5.0	60	5	96/66	705	203	14	5.3
				100/68	672	179	14	4.4		15	100/72	286	93	17	0.6
										30	95/70	322	111	17	2.2
										50	98/70	322	106	16	2.1

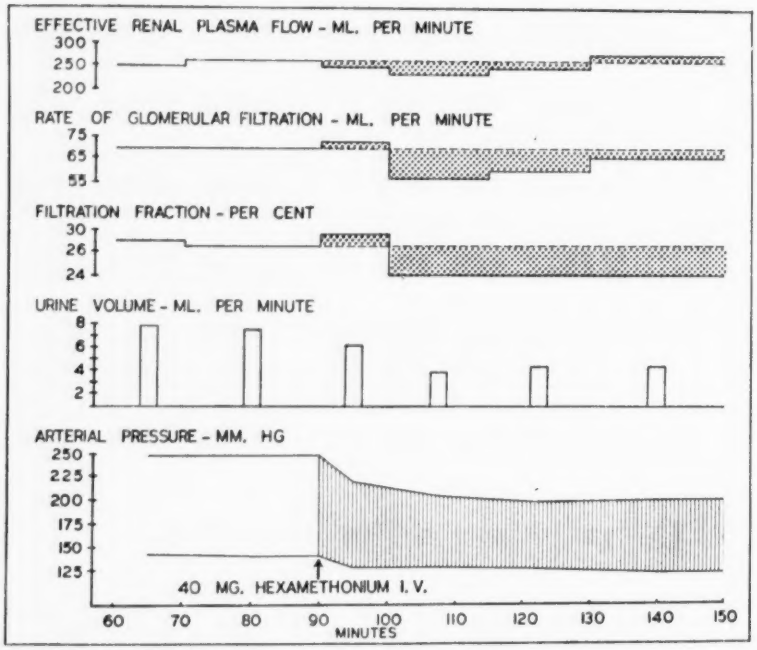


FIG. 2. CHART OF EFFECTIVE RENAL PLASMA FLOW, GLOMERULAR FILTRATION RATE, FILTRATION FRACTION, URINE VOLUME AND ARTERIAL PRESSURE BEFORE AND AFTER THE INTRAVENOUS ADMINISTRATION OF 40 MG. OF HEXAMETHONIUM ION IN PATIENT A. Z.

In the early period there was a decrease in renal plasma flow, glomerular filtration rate and urine volume. However, 45 minutes after hexamethonium renal plasma flow had returned to and glomerular filtration rate approached control values despite continued significant reduction in filtration fraction, urine volume and arterial pressure.

III. Other Aspects of Renal Function

Glomerular filtration rate was determined in six of the subjects listed in Table IV. In three hypertensive patients there was a reduction of glomerular filtration rate followed by a rise toward but not to the control values (A. Z., A. S., and M. Y., Table IV and Figure 2). In one hypertensive and one normal subject there was a continued fall in glomerular filtration rate throughout the experimental period. The final normal subject who had exhibited no significant change in arterial pressure or renal blood flow also showed no change in glomerular filtration rate. It was apparent, therefore, that the rate of glomerular filtration decreased paralleling the blood pressure fall and complete compensation usually did not occur during the experimental period. The filtration fraction decreased in all of the hypertensive patients. In the two normal subjects there was no significant change in the patient who showed no reduction of arterial pressure and a slight rise in filtration fraction in the other.

The volume of urine flow did not change in the two cases who exhibited insignificant reduction of arterial pressure (D. G. and H. B., Table IV). Four of the five remaining cases exhibited a reduction of urine volume which persisted throughout the experimental period. The final patient (C. S.) exhibited a slight rise in urine volume.

DISCUSSION

Previous hemodynamic studies in this laboratory demonstrated that hexamethonium produces a marked to complete abolition of such vasoconstrictor reflexes as the vasopressor "overshoot" following the Valsalva maneuver, the cold pres-

sor response and the reflex vasoconstrictions to "noxious" stimuli as determined by digital plethysmography (15). As would be expected following such inhibition of moderator reflexes by a ganglionic blocking agent the pressor responses to epinephrine and norepinephrine were augmented considerably, after as compared to before, hexamethonium (16). In a constant temperature cold room the increase in foot blood flow was as great following hexamethonium as after extradural or intrathecal anesthesia in normal subjects, suggesting nearly complete ganglionic blockade in man (14). In addition, probably by reason of the inhibition of vasoconstrictor reflexes, hexamethonium was found to induce marked hypotension in normal or hypertensive subjects following minor degrees of blood loss such as that occasioned by the brief application of venous tourniquets to the extremities or by small venesections of 250 to 500 cc. of blood (17). Thus, the previous studies demonstrated that hexamethonium in man interferes with transmission of sympathetic vasoconstrictor impulses more completely than any previously known agent. The present investigation has determined the effects of such widespread inhibition of sympathetic impulses on cardiovascular dynamics.

In the patients who did not exhibit congestive heart failure and whose cardiac outputs were in the normal range, the hypotensive response to hexamethonium was accompanied by a decreased cardiac output and little or no change in total peripheral resistance. These observations are in approximate agreement with those of Werkö, Frisk, Wade, and Eliasch (18), but are opposed to those of Gilmore, Kopelman, McMichael, and Milne (19) who found essentially no change in cardiac output after hexamethonium. Thus, the reduction of arterial pressure in the present series seemed to be secondary to a diminished cardiac output rather than to arteriolar vasodilation. The decreased cardiac output in turn appeared to be due to a failure of venous return since the pressures on the right side of the circulation were reduced uniformly.

The failure of venous return probably was produced by pooling of blood in the peripheral circulation. Such pooling could be due to (1) an increase in the total vascular capacity, and (2) the blockade of reflex vasoconstrictor responses (17).

By contrast in the patients with congestive heart failure or malignant hypertension the decreases in systemic and right heart pressures were accompanied by an increased cardiac output and decreased total peripheral resistance. These divergent observations in the patients with heart failure as compared to compensated subjects may be explained, however, in the light of the following considerations: first, in the case of the heart failure subjects the peripheral pooling of blood induced by hexamethonium would act like a venesection reducing the loading pressure of the congested right side of the heart thereby facilitating its recovery. Second, it is evident that patients with this type of heart failure are in a state of elevated vasoconstrictor tone since normal or elevated levels of systemic arterial pressure are maintained despite a cardiac output which usually is reduced. Hexamethonium by its blocking action on vasoconstrictor reflexes abolishes this increased tone thus permitting a decrease in total peripheral resistance and thereby also in the demand for cardiac work. These considerations and their implications in regard to the nature of congestive heart failure have been discussed more fully in another communication (20).

Muscle blood flows increased only moderately, while renal and hepatic-portal blood flows fell during the maximum action of the drug. Foot blood flow increased approximately ten fold (14) but since this vascular area is small in relation to the total vasculature the decrease in vascular resistance in the foot had little influence on the total peripheral resistance. These results suggest that there is an uneven distribution of sympathetic vasocontrictor nerves in different vascular areas. Only in a single region, the distal parts of the extremities, was a marked increase in flow observed. In the other and larger vascular areas (muscles, hepatic-portal and renal vascular beds) moderate increases or actual decreases in flow occurred while the total peripheral resistance usually was only slightly reduced. These data do not support the view that the sympathetic vasoconstrictor system is of great importance in regulating arteriolar tone in the resting, supine subject except for its role in temperature regulation of the skin particularly of the distal parts of the extremities. The results also emphasize the fallacies inherent in drawing conclusions as to the overall

vasodilating effects of agents which increase skin temperature, color or blood flow.

The hemodynamic effects of hexamethonium, however, are due to more than simple inhibition of sympathetic vasoconstrictor nerves since the drug interferes with transmission through all autonomic ganglia. Vasodilator nerves which synapse in the ganglia as well as vasoconstrictor impulses will be inhibited. For example, blockade of vasodilator impulses is seen in the cessation of salivary flow following the drug (21). Thus, the results observed after hexamethonium may be in reality the net effect of combined inhibition of autonomic vasoconstrictor and vasodilator impulses.

The pattern of change in renal function was qualitatively similar to that observed after certain other hypotensive agents including veratrum viride (9), the dihydrogenated alkaloids of ergot (22) and sodium nitrite given as a single, oral dose (23). The renal vasculature did not share in the decrease in vascular resistance associated with the fall in arterial pressure. This is indicated by the fact that the renal plasma flow decreased sharply at the onset of the hypotensive response regardless of the drug used. However, as the arterial pressure stabilized at a lower level or began to rise the resistance of the renal vessels decreased to permit a return to normal rates of plasma flow. This effect, which was first observed by Smith and his co-workers folowing spinal anaesthesia, has been interpreted by him as indicating an autonomy of the renal arterioles (24).

A recent study by Machinnon indicated that the closely related compound, pentamethonium produced a decrease in renal blood flow in both normotensive and hypertensive subjects (25). However, the design of his experiments was such that only the early hypotensive response to the drug was studied. Thus, insufficient time was allowed to permit observation of the later return of renal plasma flow to control levels. Mills, Moyer, and Handley found no change in renal clearances following hexamethonium in normal subjects but observed some diminution in hypertensive patients (26).

As a result of the percentally greater fall in glomerular filtration rate than in plasma flow there was a decrease in filtration fraction which in hypertensive subjects approached normal values. The urine during this period became quite concentrated suggesting that tubular function was not significantly impaired. This pattern of change has been observed with hypotensive agents of all types tested in this laboratory (23) and therefore probably is secondary to the sudden alteration of arterial pressure rather than to any specific action of individual drugs on the kidney.

From the point of view of effects on hemodynamics the response to hexamethonium does not appear to be entirely desirable. Thus, cardiac output may decrease, renal clearances especially glomerular filtration rate fall at least temporarily and homeostatic, vasomotor reflexes are seriously compromised. However, despite these apparently undesirable and abnormal acute actions, the clinical response to hexamethonium frequently has appeared to be beneficial (5, 7, 8). These correlative clinical and experimental studies emphasize the fact that the results of hemodynamic analysis need not always indicate the desirability of a given agent in clinical practice where other factors may determine the usefulness of the drug.

SUMMARY AND CONCLUSIONS

Hexamethonium administered to hypertensive and normal subjects produces the following hemodynamic effects:

1. In hypertensive patients who do not have cardiac decompensation the reduction of systemic arterial pressure is accompanied by a decrease in right heart pressures and cardiac output. The total peripheral resistance does not change significantly. It is suggested that these alterations are the result of a combination of "venous pooling" and failure of reflex vasoconstriction.

2. In patients with heart failure the fall in systemic arterial pressure appears to be accompanied by a reduction of right heart pressures, an increase in cardiac output and a significant decrease in total peripheral resistance. These alterations may be due to unloading of the congested right side of the heart as well as to inhibition of vasoconstrictor reflexes activated by the low output heart failure.

3. In contrast to the marked increase in blood flow in the foot observed previously, blood flow through the muscles increases only moderately. Since the arterial pressure falls after hexametho-

nium a significant decrease in peripheral resistance is assumed to occur in this area.

4. Despite a moderate reduction of hepatic vascular resistance estimated hepatic-portal blood flow usually decreases after hexamethonium.

5. Renal plasma flow decreases paralleling the initial fall in arterial pressure and then rises to approximate control levels despite a continued hypotensive response. This is consistent with previous observations indicating an autonomy of tone of the renal arterioles.

6. In most cases the changes in glomerular filtration rate follow a pattern similar to the alterations in renal plasma flow, but occasionally filtration may remain below control values. Oliguria with increased concentration of urine usually occurs. All of these renal effects begin to diminish after 30 to 60 minutes despite continued significant hypotension.

7. The studies to date suggest that sympathetic vasoconstrictor nerves blocked by hexamethonium exert the controlling influence on homeostatic adjustments to postural change as well as in maintaining the tone of the vessels of the distal part of the extremities. However, in resting supine subjects such nerves appear to exert a much less important influence on arteriolar tone in the hepatic-portal, renal and muscle areas.

ACKNOWLEDGMENT

The authors wish to thank Miss Jean Pietras for valuable technical assistance.

REFERENCES

1. Barlow, R. B., and Ing, H. R., Curare-like action of polymethylene bisquaternary ammonium salts. Nature, 1948, **161**, 718.
2. Paton, W. D. M., and Zaimis, E. J., Clinical potentialities of certain bisquaternary salts causing neuromuscular and ganglionic block. Nature, 1948, **162**, 810.
3. Organe, G., Paton, W. D. M., and Zaimis, E. J., Preliminary trials of bistrimethylammonium decane and pentane diiodide (C10 and C5) in man. Lancet, 1949, **1**, 21.
4. Arnold, P., and Rosenheim, M. L., Effect of pentamethonium iodide on normal and hypertensive persons. Lancet, 1949, **2**, 321.
5. Restall, P. A., and Smirk, F. H., The treatment of high blood pressure with hexamethonium iodide. New Zealand M. J., 1950, **49**, 206.
6. Freis, E. D., Methonium compounds in hypertension. Letter to the editor. Lancet, 1951, **1**, 909.
7. Freis, E. D., Finnerty, F. A., Jr., Schnaper, H. W., and Johnson, R. L., The treatment of hypertension with hexamethonium. Circulation, 1952, **5**, 20.
8. Finnerty, F. A., Jr., and Freis, E. D., Clinical appraisal of hexamethonium (C6) in peripheral vascular diseases. New England J. Med., 1951, **245**, 325.
9. Freis, E. D., Stanton, J. R., Culbertson, J. W., Litter, J., Halperin, M. H., Burnett, C. H., and Wilkins, R. W., The hemodynamic effects of hypotensive drugs in man. I. Veratrum viride. J. Clin. Invest., 1949, **28**, 353.
10. Hickam, J. B., and Frayser, R., Spectrophotometric determination of blood oxygen. J. Biol. Chem., 1949, **180**, 457.
11. Green, H. D., Lewis, R. N., Nickerson, N. D., and Heller, A. L., Blood flow, peripheral resistance and vascular tonus, with observations on the relationship between blood flow and cutaneous temperature. Am. J. Physiol., 1944, **141**, 518.
12. Böger, A., and Wezler, K., Die Bestimmung des arteriellen Gasamtwiderstandes am Menschen. Arch. f. exper. Path. u. Pharmakol., 1937, **186**, 43.
13. Pitts, R. F., Lotspeich, E. D., Schiess, W. A., and Ayer, J. L., Renal regulation of acid-base balance in man. I. Nature of the mechanism for acidifying urine. J. Clin. Invest., 1948, **27**, 48.
14. Schnaper, H. W., Johnson, R. L., Tuohy, E. B., and Freis, E. D., The effect of hexamethonium as compared to procaine or metycaine lumbar block on the blood flow to the foot of normal subjects. J. Clin. Invest., 1951, **30**, 786.
15. Finnerty, F. A., and Freis, E. D., Experimental and clinical evaluation in man of hexamethonium (C6), a new ganglionic blocking agent. Circulation, 1950, **2**, 828.
16. Freis, E. D., MacKay, J. C., and Oliver, W. F., The effect of "sympatholytic" drugs on the cardiovascular responses to epinephrine and norepinephrine in man. Circulation, 1951, **3**, 254.
17. Freis, E. D., Stanton, J. R., Finnerty, F. A., Jr., Schnaper, H. W., Johnson, R. L., Rath, C. E., and Wilkins, R. W., The collapse produced by venous congestion of the extremities or by venesection following certain hypotensive agents. J. Clin. Invest., 1951, **30**, 435.
18. Werkö, L., Frisk, A. R., Wade, G., and Eliasch, H., Effect of hexamethonium bromide in arterial hypertension. Lancet, 1951, **2**, 470.
19. Gilmore, H. R., Kopelman, H., McMichael, J., and Milne, I. G., The effect of hexamethonium bromide on the cardiac output and pulmonary circulation. Lancet, 1952, **2**, 898.
20. Kelley, R. T., Freis, E. D., and Higgins, T. F., The effects of hexamethonium on certain manifestations of congestive heart failure. Circulation, 1953, **1**, 169.

21. Freis, E. D., Unpublished observations.
22. Freis, E. D., Stanton, J. R., Litter, J., Culbertson, J. W., Halperin, M. H., Moister, F. C., and Wilkins, R. W., The hemodynamic effects of hypotensive drugs in man. II. Dihydroergocornine. J. Clin. Invest., 1949, **28**, 1387.
23. Freis, E. D., Stanton, J. R., Litter, J., Culbertson, J. W., Halperin, M. H., Moister, F. C., and Wilkins, R W., Hemodynamic effects of hypotensive agents in man. Am. J. Med., 1949, **7**, 414.
24. Smith, H. W., Rovenstine, E. A., Goldring, W., Chasis, H., and Ranges, H. A., The effects of spinal anesthesia on the circulation in normal, unoperated man with reference to the autonomy of the arterioles, and especially those of renal circulation. J. Clin. Invest., 1939, **18**, 319.
25. Machinnon, J., Effect of hypotension-producing drugs on the renal circulation. Lancet, 1952, **2**, 12.
26. Mills, L. C., Moyer, J. H., and Handley, G. A., Effect of hexamethonium (C6) on renal hemodynamics. Laboratory and clinical evaluation. Am. J. Med., 1952, **13**, 103.

THE DIRECT MEASUREMENT OF THE EXTRACELLULAR PHASE OF TISSUES [1, 2]

By GEORGE NICHOLS, JR.,[3] NANCY NICHOLS, WILLIAM B. WEIL,[4] AND WILLIAM M. WALLACE

(From the Departments of Pediatrics and Medicine, Harvard Medical School, and the Children's Medical Center, Boston, Mass.)

(Submitted for publication May 7, 1953; accepted August 25, 1953)

Measurements of the extracellular fluid in experimental animals and man, using inulin or thiosulfate, often result in volumes of extracellular fluid that are smaller than are compatible with balance data or tissue analysis (1). The total volume of extracellular fluid, as measured by these substances, is also frequently smaller than the extracellular fluid as measured by the volume of distribution of chloride. In an attempt to explain these discrepancies, the distribution of a single injection of thiosulfate and inulin has been measured simultaneously in the whole body and in certain tissues of nephrectomized dogs, three and six hours following its administration. The resulting data have been interpreted in relation to the content of chloride, connective tissue, and water in the tissues examined. It appears that inulin penetrates connective tissue very slowly, as demonstrated by Kruhøffer (2), and that the entire extracellular phase is not penetrated in the length of time during which inulin space measurements are usually performed. Thiosulfate has been found to be metabolized by muscle cells. However, when the volume of distribution of inulin is corrected for the amount of connective tissue unpenetrated by this substance at a given time, and compared with the volume of distribution of chloride, corrected for the higher concentration of chloride present in connective tissue water, the two volumes approach each other closely in isolated tissues, and yield identical values for the total extracellular fluid of the body. These results are in accord with the theory of Manery, Danielson, and Hastings (3) that the extracellular compartment of the body is a connective tissue phase diluted with an ultrafiltrate of plasma.

EXPERIMENTAL PROCEDURE AND METHODS

Eight adult mongrel dogs were nephrectomized under pentobarbital anaesthesia, which was maintained throughout the six hours of the experiment. Following nephrectomy, control specimens of blood, muscle, tendon, and skin were taken. These were obtained from the following sites: Muscle from the belly of the quadriceps muscles or from the adductor muscle of the hind leg; tendon from the Achilles or patellar tendons; and skin from the abdominal wall. Body weight was determined and 8 to 10 Gm. of sodium thiosulfate in 50 to 60 ml. of distilled water was injected intravenously, followed by 4 to 5 Gm. of inulin in 40 to 50 ml. of distilled water. The exact amount of inulin, or thiosulfate, given was determined by specific gravity and direct analysis. Blood samples were taken hourly for six hours and biopsies of tissues were taken at three and six hours. The animals were sacrificed at the end of the experiment, and a final body weight obtained.

Each tissue specimen was divided into three parts and each part was promptly placed in a tared weighing bottle, stoppered, and weighed.

One portion was dried to constant weight at 100 to 105° C., ground to a powder, and extracted at least three times with a mixture of equal parts of petroleum and ethyl ether to remove the neutral fat. The dry fat-free residue was then extracted with 0.75 N HNO_3, centrifuged, and aliquots of the supernatant used for the determination of sodium, potassium, and chloride (4). Sodium and potassium analyses were carried out on a flame photometer, using lithium as an internal standard (5). Chloride was measured by a micro-modification of the method of Wilson and Ball (6). All determinations were done in duplicate.

The second portion of the specimen was finely minced and transferred quantitatively to a tared 50 ml. centrifuge tube. Thirty to forty ml. of distilled water was added and the tubes reweighed to determine the exact volume of water added. The tubes were then stoppered, agitated, and allowed to equilibrate for 18 hours in a refrigerator. Control experiments, *in vitro*, in which known amounts of thiosulfate were added to tissue homogenates, showed 95 to 100 per cent recovery of thiosulfate after

[1] This work was supported by grant number H-748 from the National Heart Institute, U. S. Public Health Service.

[2] A preliminary report of this investigation appeared in the Proceedings of the 44th Annual Meeting of the American Society for Clinical Investigation, May 1, 1952, J. Clin. Invest., 1952, **31**, 652.

[3] Present address: Peter Bent Brigham Hospital, Boston, Mass.

[4] Postdoctorate Fellow, U. S. Public Health Service.

18 hours incubation in the cold (7). No precautions were taken, either in the control experiments or in the experimental tissues, to prevent contamination with sulfur metabolizing bacteria. Following 18 hours incubation, the tubes were centrifuged, the supernatant removed, and duplicate aliquots analyzed for thiosulfate by a modification of the micro method of Newman, Gilman, and Philips (8).

The tissue remaining in the centrifuge tubes was analyzed for collagen and elastin by a modification of the method of Lowry, Gilligan, and Katersky (9).

The third portion of the specimen was used for the determination of inulin by the method of hot alkali digestion described by Ross and Mokotoff (10). This method adequately reduces the high inulinoid blank of tissues but necessitates the use of a form of inulin stable in hot alkali. Such a form of inulin was prepared and used in these studies (11). While such a procedure is perhaps not essential, it insures against the possibility of unequal rates of diffusion of the various forms of inulin.

Blood samples were centrifuged and the plasma separated immediately. Plasma water was measured by determining the wet and dry weights of volumes of plasma delivered from a calibrated micropyknometer. Plasma sodium and potassium were measured by internal standard flame photometry (5). Plasma chlorides were determined by the micro method of Van Slyke and Hiller (12). Thiosulfate was measured within four hours of sampling, using the micro method of Newman, Gilman, and Philips (8). Plasma inulin was determined by the method of Roe, Epstein, and Goldstein (13). All plasma determinations were carried out in duplicate.

DEFINITIONS

The terms "inulin space," "thiosulfate space," and "chloride space" are often used to denote the same volume of body water, which is then called the "extracellular water," regardless of the method used for its measurement. It will become apparent in this paper that the volumes of distribution of inulin, thiosulfate, and chloride may differ considerably from each other, and that, at best, they are only close approximations of a true, physiological, extracellular space—a space that represents the entire volume of body fluid that lies without the cells including cerebrospinal fluid water, the water in the lumen of the gastrointestinal tract, and the exocrine secretions. The following definitions are presented in order to clarify the terms used in this discussion and are illustrated in Figures 1, 4, and 5. Although the subdivisions of extracellular water defined here may not adhere to anatomical boundaries they appear to be distinct in terms of function.

Chloride space: That volume of water which contains all the chloride of a tissue or body at the concentration of a plasma ultrafiltrate.

Connective tissue corrected chloride space: The chloride space, corrected for the slightly higher chloride concentration that is present in the connective tissue water as compared to a plasma ultrafiltrate.

Inulin (or thiosulfate) space: That volume of water which contains all the inulin (or thiosulfate) in a tissue or body at a concentration equal to that in the plasma water.

Connective tissue corrected inulin (or thiosulfate) space: That volume of water of a tissue or body which is defined by the inulin (or thiosulfate) space, plus the volume of the connective tissue water which has not been penetrated by inulin (or thiosulfate)—as defined by tendon water penetration at the corresponding time.

Extracellular water: The sum of the following components:

a. Plasma Water.

b. Rapidly Equilibrating Water: The volume of water within a tissue or body which is extracellular, extravascular, not associated with collagen or elastin, and is assumed to equilibrate rapidly with the plasma concentration of substances like inulin.[5]

c. Connective Tissue Water: That volume of water within a tissue or whole body which is associated with the collagen and elastin of the tissue and with which substances like inulin equilibrate slowly.

CALCULATION

All calculations are based on the individual values for each dog; the averages of these results are summarized in Table I, expressed as units per kilogram of fat-free tissue or water of the fat-free tissue. For the construction of the diagrams, the values were expressed in terms of a kilogram of water of fat-free tissue.

Electrolyte concentrations in the plasma ultrafiltrates were calculated by correcting their concentration in the plasma water by a Donnan factor of 0.96.

Chloride spaces were calculated by dividing the quantity of chloride in the tissue by the plasma ultrafiltrate concentration. Inulin or thiosulfate spaces were calculated by dividing the amount in the tissue water by the concentration in the plasma water.

The connective tissue water was calculated from the quantities of collagen and elastin in the samples, using the assumption that all body connective tissue contains the same concentration of water and electrolyte as is associated with tendon (90 per cent collagen). Similarly, ligamentum nuchae (81 per cent elastin) was used to calculate the water associated with elastin in other tissues. The degree to which the connective tissue phase of a tissue equilibrated with inulin or thiosulfate at any time was considered equal to the equilibration in tendon at that time.

The connective tissue corrected spaces were calculated as follows:

For chloride: The water associated with connective tissue was calculated and the amount of chloride present in this water (at a concentration equal to that in tendon)

[5] In measurements involving the whole body, the rapidly equilibrating water includes to some extent such water as is present in the gastrointestinal tract, in the cerebrospinal fluid, and in the lumina of exocrine glands.

was subtracted from the total amount of chloride present in the tissue. The remainder of the chloride was divided by the concentration of chloride in an ultrafiltrate of plasma to give the combined volume of plasma water and rapidly equilibrating water. The sum of the plasma water, rapidly equilibrating water, and connective tissue water constitute the connective-tissue-corrected chloride space.

For inulin or thiosulfate: That fraction of the connective tissue water which was unpenetrated by inulin or thiosulfate (as defined by the penetration of tendon water at the identical time) was added to the measured inulin or thiosulfate space to give the connective-tissue-corrected inulin or thiosulfate space.

The volumes of distribution for inulin and thiosulfate in the whole body were calculated by dividing the total amount of the substance given, by the plasma water concentration at each hour. This calculated space in liters of water was expressed as per cent of body weight at zero, three and six hours. The zero hour plasma levels were obtained by extrapolation from the plasma concentration curves—for thiosulfate between the first and third hours, for inulin between the third and fifth hours.

RESULTS

The averages of the analyses on the animals are presented in Table I, in terms of concentration/Kg. both of fat-free tissue and of water of fat-free tissue.

Figure 2 compares the size of the chloride space in muscle, tendon and skin water: The values used in the figure were calculated from the average of the three- and six-hour specimens of tissue. In the tendon it will be noted that the chloride space is represented as occupying more than a kilogram

TABLE I

Average tissue and serum values of eight nephrectomized dogs

Tissue	Values/Kg. fat-free tissue								Values/Kg. fat-free tissue water						
	Na	K	Cl	H_2O	Inulin*	Thio.	Collagen dry	Elastin dry	Na	K	Cl	Inulin space	Thio. space	Collagen H_2O	Elastin H_2O
	mEq.	*mEq.*	*mEq.*	*ml.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*mEq.*	*mEq.*	*mEq.*	*ml.*	*ml.*	*ml.*	*ml.*
Control Muscle	29.3	81.6	20.1	782	0	0	23.7	3.8	37.4	104.4	25.7	0	0	66.4	9.1
3 Hour Muscle	31.7	78.6	19.9	776	.1178	.0954	24.4	4.5	40.9	101.4	25.6	109.7	140.2	68.8	10.8
6 Hour† Muscle	25.6	90.2	15.6	770	.0904	.0566	10.3	4.1	33.3	117.1	20.3	96.1	101.7	29.3	10.0
Control Tendon	97.8	13.7	85.9	667	0	0	300	12.8	146.5	20.5	128.8	0	0	964	36.0
3 Hour Tendon	98.4	13.1	83.6	661	.1558	.2515	287	15.6	148.9	19.8	126.5	175.0	448.9	956	44.0
6 Hour‡ Tendon	96.2	14.2	86.4	662	.2338	.3710	292	8.1	145.3	21.4	130.6	269.3	767.5	977	23.0
Control* Skin	97.2	20.6	87.4	748	0	0	148	24.1	130.0	27.6	116.8	0	0	433	60.2
3 Hour* Skin	100.8	22.2	85.7	741	.4825	.4630	164	26.6	136.0	29.9	115.6	478.5	776.6	484	67.4
6 Hour* Skin	98.5	25.3	82.4	730	.4640	.3710	146	29.0	134.9	34.7	112.9	494.0	728.9	438	74.0
Control Serum	148.1	3.73	112.2	944	0	0			156.8	3.95	118.8				
3 Hour Serum	156.9	4.04	105.7	947	1.270	0.887			165.7	4.27	111.7				
6 Hour† Serum	156.7	4.44	106.9	940	1.220	0.721			166.8	4.72	113.8				

* Averages of 5 dogs.
† Averages of 7 dogs.
‡ Averages of 6 dogs.

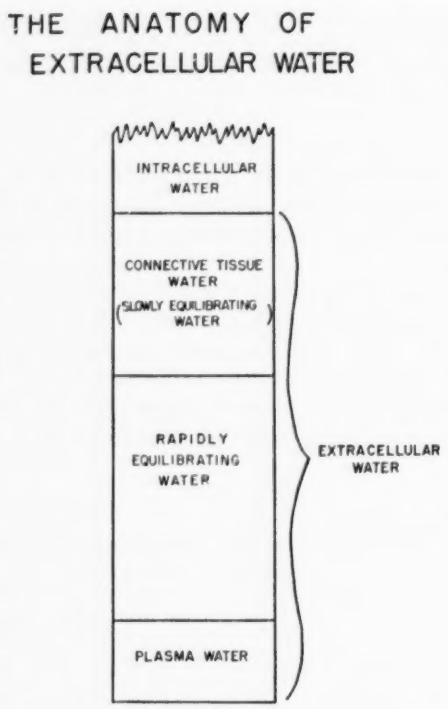


FIG. 1

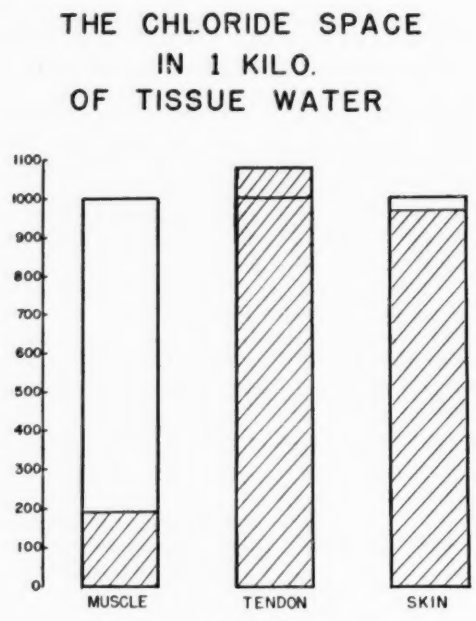


FIG. 2

of tendon water (112 per cent). This occurs because the concentration of chloride in tendon water was found to be greater than in a plasma ultrafiltrate. This has been reported previously by Manery, Danielson and Hastings (3).

Figure 3 illustrates the percentage penetration of the chloride space of the three tissues by thiosulfate and inulin at three and six hours.

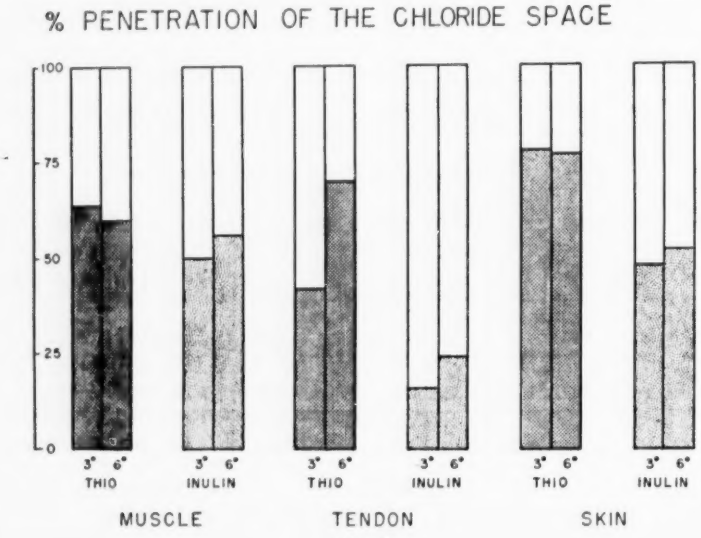


FIG. 3

Three points are apparent from Figure 3: First, in the time covered by these experiments neither thiosulfate nor inulin spaces in tissues reached the size of the chloride space. Second, thiosulfate appeared to penetrate tissues more rapidly than inulin. At both three and six hours, the thiosulfate space was larger than the inulin space. Third, in all of the tissues examined, the inulin space increased with time, whereas the thiosulfate space, although increasing in tendon, decreased in muscle and skin. This suggests that thiosulfate was broken down more rapidly in these tissues than in tendon, and that the rate of breakdown was sufficiently great to prevent the establishment of diffusion equilibrium between plasma water and the water of muscle and skin. *In vitro* experiments with muscle and tendon confirmed the fact that thiosulfate is destroyed by tissues rich in cells (7).

Figures 4 and 5 represent a kilogram of muscle and skin water at six hours and compare the results of various methods of measuring the extracellular phase water. These figures were constructed by first plotting the measured inulin space. The connective tissue water was superimposed in such a way that the portion penetrated by inulin (as calculated by the penetration of tendon at six hours) lay within the inulin space. The unpenetrated connective tissue water was placed above the inulin space. The water of the inulin space which was not connective tissue water was labelled "rapidly equilibrating water" and was assumed to be a plasma ultrafiltrate. Thus the rapidly equilibrating water plus the connective tissue water and the plasma water define an extracellular phase which has been termed the "Connective-Tissue-Corrected Inulin Space." In each case it will be noted that this estimate of extracellular water is smaller than the conventionally used chloride space. However, when the chloride space is corrected for the higher concentration of chloride which exists in collagen water as compared with plasma ultrafiltrate (*i.e.*, the Connective-Tissue-Corrected Chloride Space), the space defined by chloride becomes smaller and more nearly approaches the Connective-Tissue-Corrected Inulin Space.

Figure 6 shows the average whole body thiosulfate and inulin spaces at zero, three and six hours expressed as per cent of total body weight. At zero time the inulin space is 17 per cent and

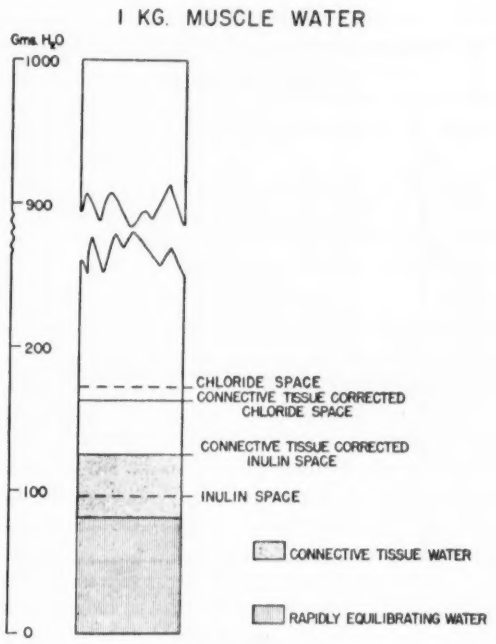


FIG. 4

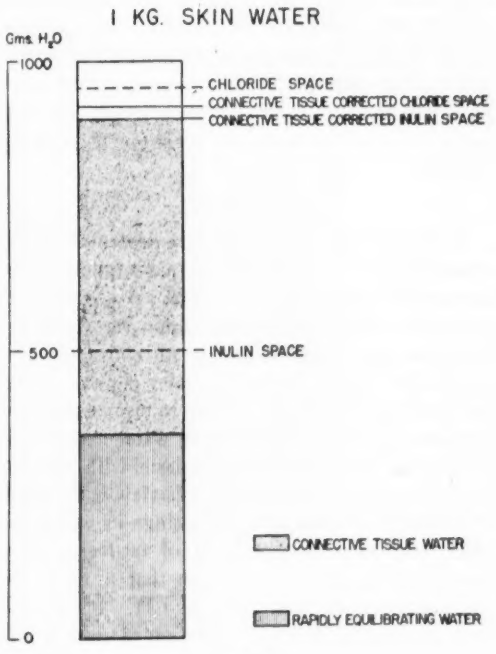


FIG. 5

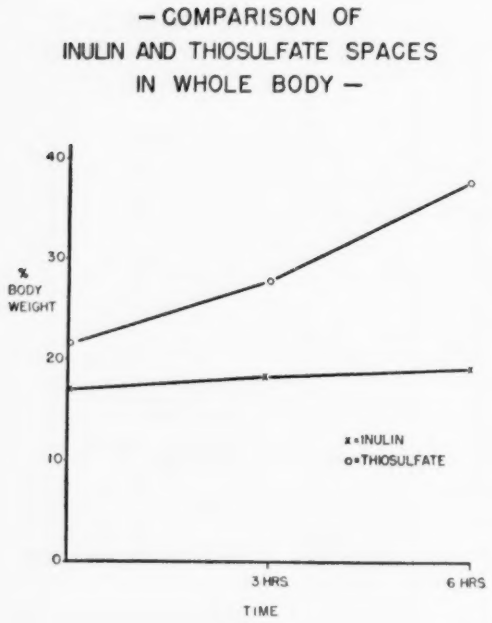


FIG. 6

the thiosulfate space 21.5 per cent; both increase with time. These values agree well with the results of other workers (14, 15, 16). The increase in the inulin space in six hours is small—amounting to 2 per cent of the body weight; on the other hand, the increase in thiosulfate space in the same period of time amounts to 16 per cent of body weight.

DISCUSSION

In the development of the data presented in this communication, a number of assumptions have been made, the validity of which may be open to question. The analysis of tendon has been assumed to be representative of all connective tissue in the body—the higher water content of tissues such as skin being assumed to represent larger amounts of rapidly equilibrating water rather than a different form of collagenous tissue with a higher water content. In support of this view is the work of Manery, Danielson and Hastings in which the water and electrolyte content of fat-free tendon was found to be identical with that of perirenal areolar tissue after fat removal (3). It has also been assumed that the penetration of tendon water by inulin or thiosulfate should reflect the penetration of all body connective tissue by these substances. Although identity in chemical composition does not necessarily impose identity in the rate of penetration by a foreign substance, the data reported here are in accord with this assumption.

The plasma disappearance curves of injected foreign substances such as inulin and thiosulfate are determined by several factors—urinary excretion, mixing through the plasma, diffusion into various components of the extracellular compartment, diffusion into body cavities and hollow viscera, and metabolic breakdown. At least two rates of disappearance are apparent from the work of Cotlove with inulin (17).

There is first a rapid rate with a half time of less than 30 minutes which probably represents mixing in the plasma and equilibration with a volume of water outside the vascular bed which reaches rapid equilibrium with plasma. The volume defined by this first rapid component probably represents the plasma and rapidly equilibrating water as defined above. The second component of the disappearance curve is very much slower, with a half time of about 10 hours. This part of the curve is usually assumed to represent equilibrium between plasma and extracellular fluid. The very slow decline of concentration that occurs in this later period is generally assigned to metabolic breakdown, pathologic shifts of water, or intracellular penetration of the substance being studied (14), but, as will be discussed, it probably represents equilibration between connective tissue water and the rest of the extracellular water. The zero hour value is extrapolated from this second component of the curve. In reality, this extrapolated value defines only the plasma and rapidly equilibrating water at a time when no connective tissue water has been penetrated.

Figure 7 shows that the slow disappearance rate of inulin can be entirely accounted for on the basis of diffusion into connective tissue water during the six hours of these experiments. This figure demonstrates the relationship between the increase with time in the inulin space of the total body and the increasing penetration of connective tissue water. The diagram is composed of four pairs of columns. The right-hand column of each pair represents the measured inulin space of a kilogram of whole body. The left-hand column is the connective-tissue-corrected inulin space of

whole body, which is seen to be composed of plasma water, rapidly equilibrating water and connective tissue water. At zero hours it is assumed that inulin has not penetrated any of the connective tissue water, and therefore defines only the plasma and rapidly equilibrating water (170 ml. per Kg. of whole body). The connective tissue water (77 ml. per Kg.) was calculated from Lightfoot and Coolidge's measurements of total body collagen (18) and our data on tendon water. The sum of plasma water, rapidly equilibrating water, and connective tissue water (or the connective-tissue-corrected inulin space) is 247 ml. per Kg. of body, and is represented by the dotted line. This figure is seen to be almost identical with figures given by other workers for the whole body chloride space (19, 20), which is represented by a dashed line.

At three hours the measured whole body inulin space is 184 ml. per Kg. of body—an increase of 14 ml. per Kg. If this increase is exclusively due to penetration of the connective tissue water, it should be equal to the product of the per cent penetration of tendon water (17.5 per cent) and the calculated amount of total body collagen water (77 ml. per Kg.). When this is calculated, a figure of 13.5 ml. is obtained. This, added to the rapidly equilibrating water plus plasma water, gives a calculated inulin space of 183.5 ml. This figure, indicated by the arrow, is almost identical with the measured value at this time (184 ml). In the diagram the fraction of connective tissue water penetrated by inulin is indicated by the cross-hatched area.

At six hours, when the measured inulin space is 191 ml., the tendon penetration is 27 per cent or 21 ml. of connective tissue water; the calculated inulin space (indicated by the arrow) is 191 ml.—identical with the measured one.

The last set of columns represents the situation at an hypothetical time (X), in excess of 24 hours, when all of the connective tissue water has been penetrated (assuming no inulin to have been destroyed by metabolic processes). The measured inulin space would therefore be equal to the connective-tissue-corrected inulin space (there being no unpenetrated connective tissue water at this time).

The studies reported here were not of sufficient duration to provide data concerning the relative sizes of the inulin and chloride spaces at 24 hours or later. Cotlove has shown that, in rats receiving a constant infusion of inulin, the inulin space in muscle continues to approach the chloride space, representing 95 per cent of it at 24 hours (17). This agrees well with the hypothesis that the in-

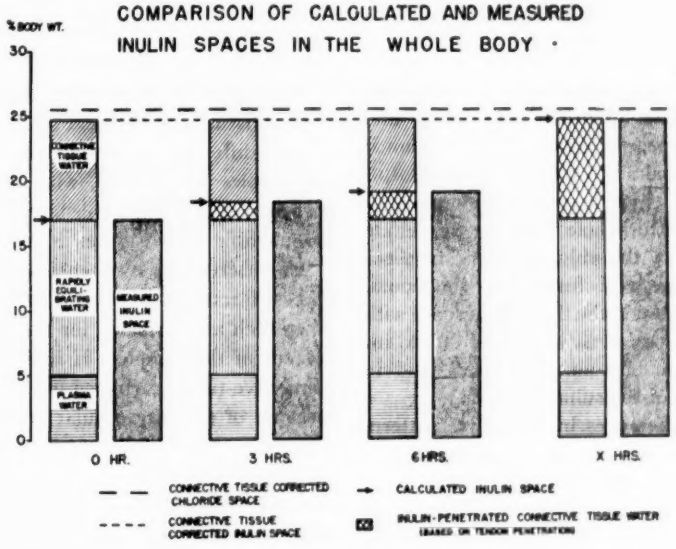


FIG. 7

crease in the inulin space with time is due to increased penetration of connective tissue. However, Finkenstaedt, O'Meara, and Merrill (21), studying the volume distribution of a single injection of inulin in anuric animals and human subjects over a period of 24 to 96 hours, showed that the inulin space increased progressively and in some cases reached a volume distribution equal to that of total body water. Other investigators were unable to recover more than 50 per cent of administered inulin in oliguric and edematous patients (22). These data suggest that inulin is metabolized in the body. In view of Cotlove's data, the rate of metabolism must be very slow, and during the six hours of the experiments described herein must have been negligible. However, it is unlikely that complete penetration of tendon water, as shown in Figure 7, ever occurs, since, by the time this state is reached, significant amounts of the injected inulin have probably been metabolized.

The increase in the thiosulfate space cannot be accounted for by increased penetration of connective tissue alone. It will be seen from Figure 7 that at zero hours the thiosulfate space is 21.5 per cent of the total body weight. By three hours the space is 27.8 per cent or 278 ml. per Kg. and exceeds the extracellular space as measured by either chloride or the connective-tissue-corrected inulin space. By six hours the space is 37.6 per cent. Since tendon is incompletely penetrated by thiosulfate at both three and six hours, correction for the unpenetrated water would further increase the space, which is already larger than is compatible with any known data. Moreover, in muscle and skin the six-hour space is actually smaller than it is at three hours, and at no time does the thiosulfate space equal the chloride space in any tissue, in spite of the fact that in whole body the thiosulfate space is larger than the chloride space at three hours and increases still further at six hours. Therefore, the increase in the thiosulfate space with time in the whole body must in part be due to increasing penetration of connective tissue and in part to destruction of thiosulfate (16, 23).

Despite small differences in electrolyte pattern, connective tissue water is similar to a plasma ultrafiltrate and distinctly different in chemical composition from intracellular water as exemplified by the analysis of highly cellular tissues such as muscle. Since, morphologically, connective tissue is largely made up of collagen and elastin fibers and very few cells, it seems more logical, as suggested by Manery, Danielson, and Hastings (3), to consider such tissues as part of the extracellular phase of the body than to arbitrarily assign them to the cellular compartment. Experimental alterations of plasma electrolyte concentrations appear to be accompanied by similar changes in the electrolyte concentrations in connective tissue water (24–26). One interpretation of these data is that the ions of connective tissue are in diffusion equilibrium with plasma, unlike the relationship which exists between muscle cells and extracellular fluid. However, more complete and conclusive data must be obtained before this equilibrium becomes accurately defined. The relationship between the total extracellular fluid as defined here and the rapidly equilibrating, physiologically active water may vary with time, with changes in acid-base balance, endocrine status, and with the state of hydration of the individual.

From the foregoing data, it would appear that a definite division of tissue water into extra- and intracellular phases cannot be made on the basis of an inulin, thiosulfate or chloride determination alone. In the nonedematous animal, the discrepancy between the extracellular phase and the space defined by a large molecule such as inulin appears to be due to the relatively slow penetration of connective tissue by this material; in the case of thiosulfate it is in part due to this slow penetration and in part due to the breakdown in tissues.

Although the incomplete penetration of connective tissue by inulin and thiosulfate makes only a small difference in the analysis of selected muscle samples (Figure 4), when applied to tissues such as skin or to the whole body the error becomes considerable (see Figures 5 and 7).

The discrepancy between the chloride and inulin spaces in tissue samples can be largely eliminated when both are corrected for the connective tissues content of the sample. If it is assumed that the connective-tissue-corrected inulin space is the true measure of the extracellular space, the remaining difference, which amounts to 1 to 3 per cent of the tissue, may represent either intracellular chloride at a concentration of less than five mEq. per L. of intracellular fluid, red cell chloride, or analytic error. Thus, either the connective-tissue-corrected

inulin space or the connective-tissue-corrected chloride space represents the best available estimate of extracellular water in individual tissues.

Both of these measurements require a knowledge of the connective tissue content of the material under study. In the case of tissue biopsies this is easily obtained; but in the whole body it is obviously impossible, although estimates are available from the carcass analysis of normal animals. While such estimates may be suitable for normals, they may be grossly in error in certain pathologic states. However, since the correction of the chloride space for the connective tissue in the whole body and for the chloride known to be present in red cells, gastric mucosa, testis, and exocrine secretions is much smaller than that required for the inulin space and further does not vary with time, the chloride space appears to be the most useful measure of the extracellular space of the whole body.

SUMMARY AND CONCLUSIONS

1. The distribution of inulin and thiosulfate has been studied simultaneously in the tissues and whole bodies of eight nephrectomized dogs and correlated with the chloride and connective tissue content of tissues.

2. In all tissues studied the chloride space exceeded the inulin and thiosulfate spaces throughout the period of study (six hours).

3. When the chloride and inulin spaces of tissues were corrected for the connective tissue content of the samples, these spaces were in close agreement.

4. In the whole body both inulin and thiosulfate spaces increased with time. The increase in the inulin space could be accounted for by the increasing penetration of connective tissue; the increase in the thiosulfate space was far greater than could be accounted for by the penetration of connective tissue, and must have been due to the destruction of this substance by cellular tissues.

5. Evidence is presented to indicate that the extracellular fluid consists of two phases: one, the total phase, measured by chloride, and a second, smaller, and probably physiologically more active one, measured by the volume distribution of large molecules such as inulin.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Miss Antoinette de Simone.

REFERENCES

1. Wallace, W. M., Some aspects of the chemical composition, physiology, and pathology of intracellular fluid. Pediatrics, 1952, **9**, 141.
2. Kruhøffer, P., Inulin as indicator for extracellular space. Acta physiol. Scandinav., 1946, **11**, 16.
3. Manery, J. F., Danielson, I. S., and Hastings, A. B., Connective tissue electrolytes. J. Biol. Chem., 1938, **124**, 359.
4. Lowry, O. H., and Hastings, A. B., Histochemical changes associated with aging: I. Methods and calculations. J. Biol. Chem., 1942, **143**, 257.
5. Berry, T. W., Chappell, D. G., and Barnes, R. B., Improved method of flame photometry. Indust. & Engin. Chem. (Analyt. Ed.), 1946, **18**, 19.
6. Wilson, D. W., and Ball, E. G., A study of the estimation of chloride in blood and serum. J. Biol. Chem., 1928, **79**, 221.
7. Nichols, G., and Nichols, N., Unpublished data.
8. Newman, E. V., Gilman, A., and Philips, F. S., Renal clearance of thiosulfate in man. Bull. Johns Hopkins Hosp., 1946, **79**, 229.
9. Lowry, O. H., Gilligan, D. R., and Katersky, E. M., The determination of collagen and elastin in tissues, with results obtained in various normal tissues from different species. J. Biol. Chem., 1941, **139**, 795.
10. Ross, G., and Mokotoff, R., Determination of inulin in muscle. J. Biol. Chem., 1951, **190**, 659.
11. Weil, W. B., Preparation of a stable form of inulin for tissue analysis. Proc. Soc. Exper. Biol. & Med., 1952, **80**, 103.
12. Van Slyke, D. D., and Hiller, A., Application of Sendroy's iodometric chloride titration to protein-containing fluids. J. Biol. Chem., 1947, **167**, 107.
13. Roe, J. H., Epstein, J. H., and Goldstein, N. P., A photometric method for the determination of inulin in plasma and urine. J. Biol. Chem., 1949, **178**, 839.
14. Gaudino, M., and Levitt, M. F., Inulin space as a measure of extracellular fluid. Am. J. Physiol., 1949, **157**, 387.
15. Schwartz, I. L., Schachter, D., and Freinkel, N., The measurement of extracellular fluid in man by means of a constant infusion technique. J. Clin. Invest., 1949, **28**, 1117.
16. Cardozo, R. H., and Edelman, I. S., The volume distribution of sodium thiosulfate as a measure of the extracellular fluid space. J. Clin. Invest., 1952, **31**, 280.
17. Cotlove, E., Inulin and chloride space in muscle. Federation Proc., 1952, **11**, 28.

18. Lightfoot, L. H., and Coolidge, T. B., The distribution of collagen in the guinea pig. J. Biol. Chem., 1948, **176**, 477.
19. Winkler, A. W., Elkinton, J. R., and Eisenman, A. J., Comparison of sulfocyanate with radioactive chloride and sodium in the measurement of extracellular fluid. Am. J. Physiol., 1943, **139**, 239.
20. Harrison, H. E., Darrow, D. C., and Yannet, H., The total electrolyte content of animals and its probable relation to the distribution of body water. J. Biol. Chem., 1936, **113**, 515.
21. Finkenstaedt, J. T., O'Meara, M. P., and Merrill, J. P., Failure of equilibration of inulin in anuric subjects. J. Clin. Invest., 1952, **31**, 627.
22. Last, J. H., Jones, R. A., McDonald, G. O., and Bond, E. E., Failure to quantitatively account for infused inulin in human subjects. Federation Proc., 1952, **11**, 367.
23. Gilman, A., Philips, F. S., and Koelle, E. S., The renal clearance of thiosulfate with observations on its volume distribution. Am. J. Physiol., 1946, **146**, 348.
24. Muntwyler, E., Mellors, R. C., Mauntz, F. R., and Mangun, G. H., Electrolyte and water equilibrium in the dog. III. Electrolyte and water exchange between tendon and blood. J. Biol. Chem., 1940, **134**, 389.
25. Amberson, W. R., Nash, T. P., Mulder, A. G., and Binns, D., The relationship between tissue chloride and plasma chloride. Am. J. Physiol., 1938, **122**, 224.
26. Nichols, N., Unpublished data.

THE BODY/VENOUS HEMATOCRIT RATIO: ITS CONSTANCY OVER A WIDE HEMATOCRIT RANGE

By H. CHAPLIN, Jr.,[1] P. L. MOLLISON and H. VETTER[2]

(From the Medical Research Council's Blood Transfusion Research Unit and Radiotherapeutic Research Unit, Postgraduate Medical School of London, London, England)

(Submitted for publication June 18, 1953; accepted July 23, 1953)

Most observers have found that when circulating red cell volume is deduced from measurement of plasma volume and venous hematocrit a higher value is obtained than when the red cell volume is measured directly. It is widely believed that this is because the proportion of red cells in the blood in large vessels is higher than that in the body as a whole and various correction factors have been proposed. Some confusion arises because the venous hematocrit must be corrected on two accounts: firstly because, under standard conditions of centrifugation, some plasma is trapped in the red cell column of the hematocrit and thus the uncorrected reading over-estimates the proportion of red cells in the sample; when this correction has been applied one may speak of the "true" venous hematocrit. Secondly, this corrected venous hematocrit value still over-estimates the proportion of red cells in the circulating blood as a whole and must be multiplied by a factor of approximately 0.9 to give an estimate of "body hematocrit." Although these concepts are accepted by the majority of investigators, the precise value of the ratio $\frac{\text{body hematocrit}}{\text{venous hematocrit}}$ is in doubt; moreover it has been suggested that the ratio may be inconstant so that it cannot safely be used to deduce blood volume from an estimate of either red cell volume or plasma volume. Accordingly, it is generally held that the only reliable estimates of total blood volume are those based on separate measurements of both red cell volume and plasma volume.

If, in fact, the body/venous hematocrit ratio appeared to be inconstant, the inconstancy could arise either from a true physiological variation between different subjects, or from the effect of abnormal states of the circulation, or it might depend solely on some systematic error in the measurement of either red cell volume or plasma volume. If the observed difference between the body hematocrit and the venous hematocrit is due to the fact that blood in the small capillary vessels has a very much lower hematocrit than blood in large vessels, it is possible to conceive of only a very few conditions (see Discussion) in which a variation in the body/venous hematocrit ratio could be expected to occur. If on the other hand the observed discrepancy between body hematocrit and venous hematocrit depends upon a systematic error in measuring, for example, plasma volume, it can be calculated that the discrepancy should vary with the level of venous hematocrit. In fact very few observers have estimated the body/venous hematocrit ratio over a sufficiently wide range to test this. Mollison, Veall, and Cutbush (1) reported measurements in newborn infants covering the observed venous hematocrit range 19 to 70 per cent; they found the ratio to be constant over this range although the absolute value was a little lower than that previously described for normal adults. Similar studies in adults, over the hematocrit range 25 to 72 per cent have been reported (2–4); however, very few anemic subjects were included and no conclusions can be drawn as to the effect of anemia or polycythemia upon the constancy of the ratio.

The present paper reports twenty-eight simultaneous plasma volume and red cell volume measurements on adult subjects with hematocrits ranging from 9 to 82 per cent. The ratio of the body hematocrit to the venous hematocrit was found to be constant throughout this range. The paper includes a discussion of various factors which determine the value assigned to the ratio.

[1] U. S. Public Health Service Research Fellow and, more recently, Senior Assistant Surgeon (R.), U. S. Public Health Service.

[2] British Council Research Fellow on leave from 2nd Medical University Clinic, Vienna.

METHODS

Subjects

Twenty-eight measurements were made on a group of nineteen subjects; two were normal volunteers, seventeen were patients (Table I). Not more than two measurements were made on the same subject; generally the second measurement was made after some major change in hematocrit had occurred—for example, in a patient with polycythemia vera, before and after radiation therapy.

Plasma volume

Plasma volume was measured using the dye T-1824 (Evans Blue). All subjects had fasted for 12 hours and had been recumbent for at least 30 minutes before the procedure. Twenty ml. of a 0.075 per cent solution of dye (Imperial Chemical Industries Batch PD/RS. 337/44) was injected intravenously from a calibrated syringe and this was followed by an injection of P^{32} labelled red cells (see below). Immediately before injection, 15 ml. of blood was withdrawn and three further 15 ml. samples were withdrawn at 15-minute intervals after injection (*i.e.*, at 15, 30, and 45 minutes). The blood was in each case taken into a syringe containing a few drops of liquid heparin and mixed in the syringe; 5 ml. was put into a tube for measurement of radio-activity (see below) and the remaining 10 ml. was delivered gently, without bubbling, into a dry test tube. A sample was removed immediately for determination of packed cell volume (observed venous hematocrit). Any blood remaining on the walls of the test tube was removed on a cotton swab, slightly moistened with saline solution. The tube was then tightly capped and centrifuged at 3000 r.p.m. for approximately 40 minutes. After centrifugation the plasma was transferred to another dry test tube and kept at room temperature for not more than 3 to 4 hours before being used for colorimetric analysis.

Approximately 0.02 ml. of the injection solution was diluted in 3 ml. of pre-injection plasma, delivered from a calibrated 3 ml. pipette. The volume of injection solution was chosen to give a concentration of the dye in plasma essentially the same as that found in the initial post-injection blood sample. The small volume of injection solution was delivered from an "Agla" micrometer-syringe, with which very satisfactory precision can be attained (5).

All samples were read in a photo-electric colorimeter using a deep red filter (combination of Ilford 803 and 205; [1]).

When the dye concentrations were plotted against time it was evident that there was some variability in the dye disappearance curves in different cases; furthermore, it was often difficult to decide the best possible straight line to be drawn through the three points. It was considered that these variations and difficulties were largely the results of technical error and did not necessarily represent true variations in the individual subjects. Accordingly, the dye disappearance rates in all twenty-eight determinations were combined and a mean loss rate of 10 per cent per hour was found. In determining the dye concentration at zero time in each subject, the values for the 15, 30 and 45-minute blood samples (mean—30 minutes) were averaged and corrected for a 5 per cent loss of dye. Plasma volume was then calculated in the usual way by comparing the dye concentration at zero time with the dye concentration of the standard.

Red cell volume

Red cell volume was measured using a modification (6) of the radio-active phosphorus method described by Reeve and Veall (7). With the modified method, in a series of duplicate determinations on ten normal males, the standard error of the difference between duplicate measurements was found to be 58 ml. (a coefficient of variation of 2.7 per cent) (6). In preparing the radio-actively tagged cells for injection, the cells were washed in a mixture of 3 per cent of plasma in ice-cold saline (6); the "extracellular" radio-activity of the injection suspension was measured and was in all cases less than 1 per cent of the total activity of the suspension.

The washed P^{32}-tagged red cells were injected about 30 seconds after completion of the injection of Evans Blue dye. Five ml. aliquots from the 15, 30, and 45-minute blood samples were used for the analyses of radio-activity.

The body hematocrit

The body hematocrit was calculated from:

$$\frac{\text{Red Cell Volume}}{\text{Red Cell Volume plus Plasma Volume}}$$

The venous hematocrit

The packed cell volume was determined using Wintrobe hematocrit tubes and a centrifuge having a radius of 15 cm. from the spindle centre to the bottom of the hematocrit tube. The tubes were spun for 30 or 55 minutes at 3000 r.p.m.; the longer time was used in the later cases after it had been shown that more consistent results could thus be obtained (8). The observed packed cell volume (excluding the buffy coat) was corrected for plasma trapped in the red cell column, using the calibration curve appropriate to 30 minutes or 55 minutes centrifugation (8). The corrected values are described as the "true" venous hematocrit.

Packed cell volumes were determined on the pre-injection sample and on the three post-injection samples. In calculating the body/venous hematocrit ratio, the true venous hematocrit was taken as the mean of the true packed cell volumes on all four blood samples.

RESULTS

The results of a comparison of the body hematocrit with the true venous hematocrit (*i.e.*, observed venous hematocrit corrected for trapped plasma) are shown in Table I, with various other data. The mean value for the body/venous he-

TABLE I

The relationship of the body hematocrit to the venous hematocrit in twenty-eight adult subjects

Subject	Sex	Age (*yrs.*)	Weight (*Kg.*)	Height (*cm.*)	Diagnosis	Venous hematocrit (mean of 4 samples) Observed (%)	Venous hematocrit (mean of 4 samples) "True" (%)	R.C.V. P32 (*ml.*)	P.V. Evans blue (*ml.*)	Body hematocrit (%)	Body hematocrit / True venous hematocrit
1*	F	64	50.9	155	Myelosclerosis	9.1	8.7	426	4880	8.0	0.926
2	F	45	52.8	167	Hemolytic Anemia	12.2	11.9	359	2920	11.0	0.924
3	F	52	52.3	154	Menorrhagia	18.1	17.4	602	3213	15.8	0.909
4	F	25	47.3	160	Hemolytic Anemia	22.9	22.1	728	2933	19.9	0.900
5	F	51	64.1	169	Polycythemia Vera (treated)	29.1	28.1	1519	3949	27.8	0.989
6	F	45	57.3	166	Arterio-venous Aneurysm	31.0	29.8	1073	2931	26.8	0.900
7	F	25	47.3	160	Hemolytic Anemia	31.3	30.0	1056	2760	27.7	0.923
8	F	35	49.2	153	Post-partum Anemia	32.0	30.8	1105	2862	27.9	0.906
9	F	35	49.2	153	Post-partum Anemia	36.8	35.5	1173	2494	32.0	0.901
10	F	31	67.8	152	Adrenal Tumor	40.3	38.4	1296	2450	34.6	0.901
11	F	67	51.8	152	Polycythemia Vera (treated)	40.7	39.8	1366	2397	36.3	0.912
12*	F	67	47.8	152	Polycythemia Vera (treated)	45.3	43.3	1691	2403	41.3	0.954
13	M	26	63.2	178	Normal Male	45.7	44.5	2300	3590	39.1	0.878
14	M	26	63.2	178	Normal Male	46.2	45.1	2293	3467	39.8	0.883
15	M	54	62.3	194	Polycythemia Vera (treated)	47.6	45.6	2289	3205	41.7	0.915
16*	F	54	52.8	163	Polycythemia Vera (treated)	48.6	46.3	1740	2392	42.1	0.909
17	M	30	80.0	187	Normal Male	47.5	46.4	2140	3060	41.2	0.888
18	M	30	80.0	187	Normal Male	49.2	48.0	2243	2900	43.6	0.909
19*	M	74	42.8	173	Polycythemia Vera (treated)	54.1	51.6	2813	3238	46.5	0.901
20*	M	50	71.8	183	Polycythemia Vera (treated)	54.9	53.5	4283	4300	49.9	0.933
21*	M	50	67.9	183	Polycythemia Vera	57.1	54.4	4432	4920	47.4	0.871
22*	F	65	75.0	173	Polycythemia Vera	62.4	59.0	3758	3635	50.9	0.862
23*	M	54	59.5	194	Polycythemia Vera	64.6	61.1	3724	2950	55.9	0.914
24*	F	65	70.0	173	Polycythemia Vera (treated)	63.4	61.5	3972	3508	53.1	0.863
25*	F	60	43.7	157	Polycythemia Vera	71.8	67.8	3197	1903	62.7	0.924
26	F	63	61.0	166	?Polycythemia Vera Uterine Tumor	73.7	69.6	3713	2048	64.5	0.926
27*	M	70	51.0	173	Polycythemia Vera	77.3	73.1	4325	2037	68.0	0.930
28*	M	74	46.7	173	Polycythemia Vera	82.3	77.6	5890	2355	71.4	0.920
											Mean = 0.910 (0.9098)
											S.D. = 0.026

* Moderate or marked splenomegaly at the time of blood volume measurement.

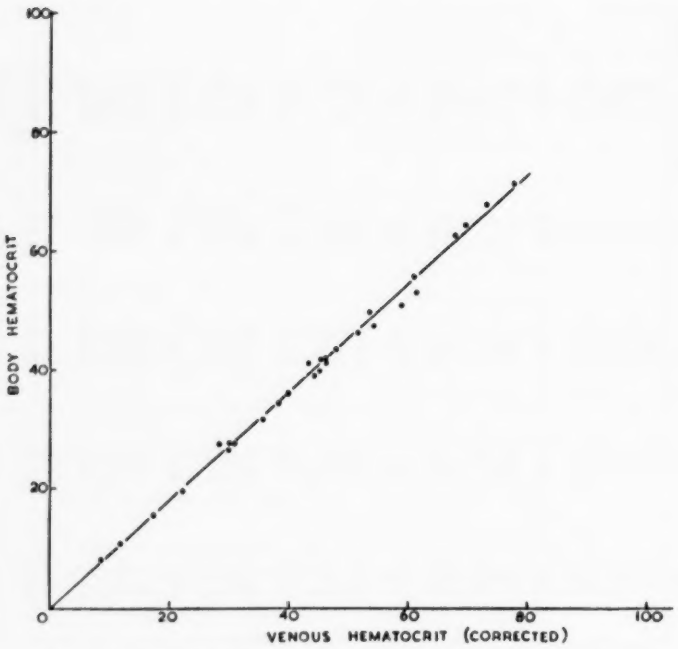


FIG. 1. ESTIMATES OF BODY HEMATOCRIT, BASED ON TWENTY-EIGHT SIMULTANEOUS MEASUREMENTS OF RED CELL VOLUME AND PLASMA VOLUME, PLOTTED AGAINST OBSERVED VENOUS HEMATOCRIT, CORRECTED FOR TRAPPED PLASMA

The line is drawn from the regression equation: BH = 0.9073 VH + 0.04, calculated by the method of least squares from the points relating body hematocrit (BH) to venous hematocrit (VH).

matocrit ratio obtained from these data is 0.910 (0.9098), with a standard deviation of 0.026.

Measurements covered the observed hematocrit range 9 to 82 per cent and in Figure 1 the body hematocrit is plotted against the true venous hematocrit. The relationship of the body hematocrit to the venous hematocrit over this range appears to be constant, the calculated regression line passing almost exactly through the origin.

DISCUSSION

There are still some workers (9) who believe that the discrepancy between the body hematocrit and venous hematocrit may depend upon a systematic error in the measurement of plasma volume. However it is unnecessary to postulate such an error for it seems likely, on theoretical grounds alone, that a discrepancy exists. For example it has been shown that blood streaming through fine tubes has a lower hematocrit than blood in large tubes (10). In the body this phenomenon seems very likely to lead to a discrepancy between the venous hematocrit and the whole body hematocrit. The observation in the present paper that the body/venous hematocrit ratio is independent of the level of the venous hematocrit strongly suggests that there is no systematic over-estimate of plasma volume; for if there were, relatively high ratios would be obtained in polycythemic subjects and lower ratios in anemic subjects.

One piece of evidence which is valuable because it does not depend on plasma volume estimation is that from replacement transfusions. Veall and Mollison (11) found that the removal of red cells was more efficient than would have been expected if the venous hematocrit were representative of the distribution of red cells and plasma in the blood as a whole; the discrepancy could best be explained by supposing that the blood in the body as a whole

had a hematocrit value about 0.9 times that of venous blood.

Although most authors accept the concept of a body/venous hematocrit ratio, there remains some disagreement as to the value which should be assigned to the ratio (see Table II). The differences shown in Table II are not in fact significant (at the level $p = 0.05$). Nevertheless, different workers are bound to assign slightly different values to the ratio due to lack of uniformity in the interpretation of data; this may be illustrated by the following two examples.

Estimation of the true venous hematocrit. Several factors will affect the value chosen for the true venous hematocrit. Firstly, it is the practice of some authors to include the white cell layer in their reading of the observed packed cell volume; this of course results in the reporting of lower values for the body/venous hematocrit ratio than when the buffy coat is excluded. Secondly, although most authors agree that the observed packed cell volume must be corrected for trapped plasma, there is considerable variation in the correction factor which is applied (13, 14). The higher the correction factor used for the plasma trapped in the packed cell column, the higher will be the value obtained for the body/venous hematocrit ratio. A third factor of importance will be the choice of blood samples used for determining the venous hematocrit. In the present investigation, it was decided to use the mean packed cell volume of all four blood samples. However, as Reeve (15) has pointed out, when multiple small blood samples are withdrawn from a subject, there is sometimes a slight progressive fall in the packed cell volume of successive samples. A slight progressive fall in the venous hematocrit was observed in eleven of the twenty-eight determinations included in this study; however this fall was less than one hematocrit division in all but two cases. The "fall" was greatest between the pre-injection and first post-injection blood sample. Since the venous stasis preceding the withdrawal of the pre-injection sample was generally more pronounced than that preceding the post-injection samples, the "fall" in hematocrit may have been in part related to stasis. In any event, since the four blood samples generally showed very close agreement it was decided that a mean of the four values would be most representative of the venous hematocrit at the time of the blood volume measurement. Table III shows the effect upon the ratio of recalculating the data for the twenty-eight measurements, using the packed cell volume of the pre-injection blood sample alone.

TABLE II

*The body/venous hematocrit ratio in adults, according to various authors**

Reference	Number of cases	Hematocrit range	Body/Venous Hematocrit ratio Mean	(S.D.)
Gibson *et al.* (12)	40	38–49	0.906	(0.082)
Reeve and Veall (7)	13	38–47	0.915	(0.012)
Nachman *et al.* (2)	40	26–59	0.895	(0.067)
Berson and Yalow (4)	25	16–72	0.924	(0.040)
Present authors	28	9–82	0.910	(0.026)

* The mean and S.D. of some of the results (2,7) have been taken from the paper by Reeve (15). The other S.D.'s were calculated from published data.

TABLE III

Effect of deducing body/venous hematocrit ratio from different venous samples

Samples used for venous hematocrit	Body/Venous Ratio*
Pre-injection *plus* three post-injection	0.910
Pre-injection *alone*	0.903

* Mean of twenty-eight measurements.

The interpretation of dye disappearance curves. When the dye concentration of successive samples is plotted against time, the slope is found to be curvilinear, even when dye concentration is plotted on a logarithmic scale; the result of an actual experiment is shown in Figure 2. To illustrate the effects of extrapolating back to zero time from points obtained relatively soon or late after the injection of the dye, a solid line has been drawn through the points obtained within the first 40 minutes after injection of the dye and an interrupted line through the points obtained between 40 and 80 minutes after injection. The effect of these two extrapolations upon the value for the plasma volume and hence, upon the body/venous hematocrit ratio, is shown. It must be admitted that differences of the magnitude illustrated here are unlikely to occur in practice, for most workers take their blood samples within the first hour after injection.

Another method of estimating dye concentration

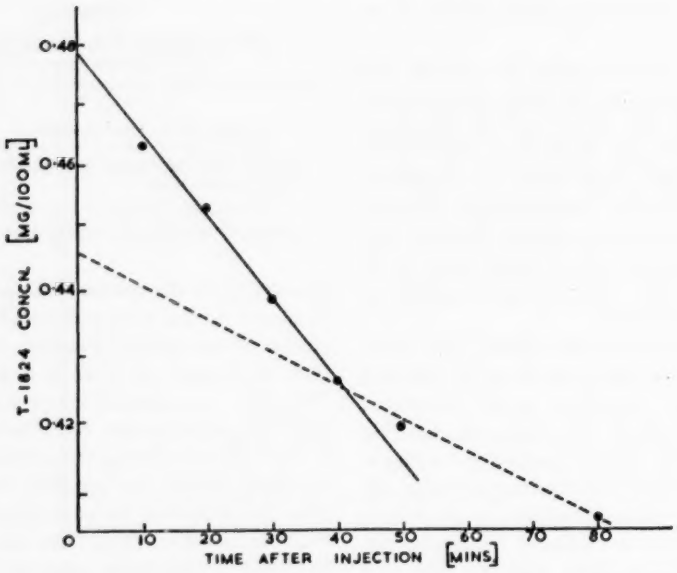


FIG. 2. OBSERVATIONS OF DYE CONCENTRATION (PLOTTED ON A LOGARITHMIC SCALE) ON A NORMAL SUBJECT DURING THE 80 MINUTES FOLLOWING AN INJECTION OF EVANS BLUE

The solid line drawn through the points obtained during the first 40 minutes led to a plasma volume estimate of 3190 ml. with a body/venous hematocrit ratio of 0.917; the dotted line drawn through the points obtained during the second 40 minutes led to a plasma volume estimate of 3420 ml. with a body/venous hematocrit ratio of 0.880.

at zero time, used for example in the present paper, is to assume a constant rate of dye loss during the first 45 minutes after injection and to apply a correction to the observed concentration rather than trying to estimate the rate of loss in each case.

It is obvious that the use of these different conventions will lead to different estimates of plasma volume and thus to different estimates of the body/venous hematocrit ratio in a given patient.

In practice most workers have found values within the range 0.90 to 0.92 in normal adults. If a worker elects not to determine a value for the body/venous hematocrit ratio under his own conditions of measurement, it seems justifiable for him to select a value within this range.

It has been mentioned that there may be a few clinical conditions in which the body/venous hematocrit ratio may actually depart significantly from the constant value described above. The rarity of these conditions is not surprising if one considers the physiological interpretation of the discrepancy between body hematocrit and venous hematocrit. It can be shown that in order to produce a body hematocrit 10 per cent lower than the venous hematocrit, assuming that 20 per cent of the total blood volume is distributed in the capillary bed (16), one must postulate a hematocrit in capillary blood which is only half as great as the hematocrit in the venous blood. It is apparent that in order to raise the body/venous hematocrit ratio significantly (say, from 0.90 to 0.95) one would require either (a) complete shutdown of half of the capillary bed, or (b) very great calibre enlargement of half the capillary bed, or (c) a reservoir of blood somewhere in the body, wherein there was circulating at least 15 per cent of the total blood volume, having a hematocrit one third higher than the venous hematocrit. There are very few clinical conditions in which these requirements

are likely to be fulfilled. For example it is likely that during prolonged oligemic shock, capillary blood flow is so radically altered as to disturb the normal body/venous hematocrit ratio. In some patients with severe anemia accompanied by massive splenomegaly, there may be a large enough volume of "concentrated" blood flowing through the spleen to produce a body/venous hematocrit ratio close to 1.0 (17). However, massive splenomegaly by itself will not raise the body/venous hematocrit ratio in the non-anemic individual, presumably because the spleen's ability to concentrate blood is limited. For example, in the present series, there were twelve patients with moderate or gross splenomegaly, only one of whom was anemic; the average body/venous hematocrit ratio in these twelve patients was 0.909, that is to say, almost precisely the same as that of the whole series. Finally, it has been suggested (18) that in patients with congestive cardiac failure, the measurement of plasma volume by the dye method may be seriously in error. If this is true it seems best in this condition to deduce total blood volume from a measurement of red cell volume alone, using a standard correction factor for the body/venous hematocrit ratio.

The fact, demonstrated clearly by the present data, that the body/venous hematocrit ratio does not vary with the venous hematocrit is of considerable importance. It implies that if a worker standardizes his conditions of measurement and determines the value for the body/venous hematocrit ratio under these conditions, he may then proceed with confidence to use this value to deduce total blood volume from a measurement either of red cell volume or plasma volume alone. Alternatively, using the conventions described in the present paper, the value of the ratio may be assumed to be 0.91.

The method of estimating blood volume is then as follows: The observed venous hematocrit is corrected for trapped plasma (8) and then multiplied by 0.91 to obtain the "body hematocrit" (BH). Blood volume is then given by:

$$\text{Red cell volume} \times \frac{100}{\text{BH}}$$

or

$$\text{Plasma volume} \times \frac{100}{100 - \text{BH}}$$

SUMMARY

The ratio $\frac{\text{body hematocrit}}{\text{venous hematocrit}}$ has been estimated in adults over the observed venous hematocrit range 9 to 82 per cent and found to be constant (twenty-eight measurements). The value for the ratio was 0.910 with a standard deviation of 0.026. It is pointed out that the exact value assigned to this ratio depends to a small extent on interpretation, but that this does not affect its usefulness in deducing blood volume from a measurement of red cell volume or plasma volume alone.

ACKNOWLEDGMENTS

We should like to thank Drs. J. D. Abbatt, J. F. Goodwin, and J. C. Hawksley and Professors J. M. McMichael and M. L. Rosenheim for allowing us to make observations upon patients under their care. Dr. C. M. Scott of Imperial Chemical Industries kindly supplied the Evans Blue dye.

REFERENCES

1. Mollison, P. L., Veall, N., and Cutbush, M., Red cell and plasma volume in newborn infants. Arch. Dis. Child., 1950, **25**, 242.
2. Nachman, H. M., James, G. W., III, Moore, J. W., and Evans, E. I., A comparative study of red cell volumes in human subjects with radioactive phosphorous tagged red cells and T-1824. J. Clin. Invest., 1950, **29**, 258.
3. Wasserman, L. R., Yoh, Tse-fei, and Rashkoff, I. A., Blood volume determination: comparison of T-1824 and P^{32} labeled red cell methods. J. Lab. & Clin. Med., 1951, **37**, 342.
4. Berson, S. A., and Yalow, R. S., The use of K^{42} or P^{32} labeled erythrocytes and I^{131} tagged human serum albumin in simultaneous blood volume determinations. J. Clin. Invest., 1952, **31**, 572.
5. Barnes, D. W. H., Loutit, J. F., and Reeve, E. B., A comparison of estimates of circulating red blood cell volume given by the Ashby marked red cell method and the T-1824-haematocrit method in man. Clin. Sc., 1948, **7**, 135.
6. Chaplin, H., Jr., Precision of red cell volume measurement using P^{32}-labelled cells. J. Physiol., In press.
7. Reeve, E. B., and Veall, N., A simplified method for the determination of circulating red-cell volume with radio-active phosphorus. J. Physiol., 1949, **108**, 12.
8. Chaplin, H., Jr., and Mollison, P. L., Correction for plasma trapped in the red cell column of the hematocrit. Blood, 1952, **7**, 1227.
9. Prentice, T. C., Berlin, N. I., Hyde, G. M., Parsons, R. J., Lawrence, J. H., and Port, S., Total red

cell volume, plasma volume, and sodium space in congestive heart failure. J. Clin. Invest., 1951, **30**, 1471.

10. Fåhraeus, R., The suspension stability of the blood. Physiol. Rev., 1929, **9**, 241.
11. Veall, N., and Mollison, P. L., The rate of red cell exchange in replacement transfusions. Lancet, 1950, **ii**, 792.
12. Gibson, J. G., 2nd, Peacock, W. C., Seligman, A. M., and Sack, T., Circulating red cell volume measured simultaneously by the radioactive iron and dye methods. J. Clin. Invest., 1946, **25**, 838.
13. Maizels, M., Intercellular plasma in the centrifuged erythrocytes of normal human blood. Quart. J. Exper. Physiol., 1945, **33**, 129.
14. Chapin, M. A., and Ross, J. F., The determination of true cell volume by dye dilution, by protein dilution, and with radioactive iron. Error of the centrifuge hematocrit. Am. J. Physiol., 1942, **137**, 447.
15. Reeve, E. B., Use of radioactive phosphorus for the measurement of red-cell and blood volume. Brit. M. Bull., 1952, **8**, 181.
16. Gibson, J. G., 2nd, Seligman, A. M., Peacock, W. C., Aub, J. C., Fine, J., and Evans, R. D., The distribution of red cells and plasma in large and minute vessels of the normal dog, determined by radioactive isotopes of iron and iodine. J. Clin. Invest., 1946, **25**, 848.
17. Verel, D., Personal communication.
18. Ross, J. F., Chodos, R. B., Baker, W. H., and Freis, E. D., The blood volume in congestive heart failure. Tr. A. Am. Physicians, 1952, **65**, 75.

BACTERIAL DIGESTION OF COLLAGEN [1]

By JOHN D. MacLENNAN, INES MANDL, AND EDWARD L. HOWES

(From the Departments of Surgery and Microbiology, College of Physicians and Surgeons, Columbia University, New York, N. Y.)

(Submitted for publication February 20, 1953; accepted August 18, 1953)

That some species of Clostridia elaborate proteolytic enzymes has been known since the earliest days of bacteriology (1–3). Indeed, it was soon realized that such organisms were unusually active in this respect and played a major part in the natural processes of putrefaction (4–8). Yet neither these studies nor the much more detailed chemical investigations undertaken during and shortly after World War I (9–13) revealed the occurrence of any specific collagenolytic activity. It is true that Henry (14) reported that culture filtrates from *Cl. perfringens* had the property of disintegrating fresh muscle-tissue, but he does not seem to have appreciated the nature of this reaction which we now know to be due to the presence of a collagenase (15). Subsequently, however, in 1931 and 1932 Weinberg and Randin (16, 17) announced that filtrates from cultures of the anaerobe *Cl. histolyticum* and the aerobe *B. anthracoides* would slowly digest small pieces of fresh Tendo Achillis. But these important observations were ignored or forgotten until interest was once more aroused by the work of Maschmann on the protein metabolism of certain anaerobic organisms. In 1937 this worker (18, 19) isolated an enzyme from *Cl. perfringens* that would digest gelatin but had no effect on several other protein substrates with the solitary exception of collagen. He suggested the name "collagenase" for this agent but subsequently (20), for some undisclosed reason, withdrew the term. Several years later, MacFarlane and MacLennan (15) reported that in filtrates of *Cl. perfringens* Type A cultures, a substance was present which would dissolve pieces of fresh Tendo Achillis and destroy the collagen framework of muscle-tissue. They produced evidence that this activity was not due to any of the known enzymes elaborated by *Cl. perfringens*, a finding shortly afterwards confirmed by Oakley, Warrack, and Van Heyningen (21) by immunological methods. This new enzyme was later definitely identified as a collagenase by Bidwell and Van Heyningen (22). Subsequent work at first seemed to indicate that essentially similar enzymes were produced by other organisms (23–27). However, it has been clearly established that other bacterial proteases exist, unable to attack "native" collagen, but more or less readily hydrolyzing collagen which has been "denatured" by various physical and chemical means (28–31, 22).

[1] This work was supported in part by a grant from the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, Contract No. DA 49-007-MD-14.

MATERIAL AND METHODS

Organisms

The majority of the strains used in the present study were obtained from the routine diagnostic service of the Surgical Bacteriology Laboratory. The three strains of Myxobacteria were kindly furnished by Dr. E. J. Ordal of the University of Washington. As for the Clostridia, many of these had been isolated by Dr. Ivan Hall during the bacteriological studies made on traumatic wounds by Dr. Frank Meleney and his co-workers during World War II. Others came from our own collected material (J. D. MacL.) and some were obtained from other sources; in particular Dr. C. L. Oakley of the University of Leeds and Dr. L. S. McClung of Indiana University.

Each organism was cultivated in a suitable fluid medium (usually nutrient broth with 1 per cent proteose peptone) for from 12 to 18 hours. A sample of the culture was then removed, clarified by centrifugation and examined for the presence of collagenolytic enzymes by the following methods.

a. "Natural" collagen: In these experiments the usual substrate was a small (approximately 5 by 3 by 2 mm.) piece of fresh rabbit Tendo Achillis. The culture sample (in various dilutions) was allowed to act at 37° C. for periods of up to 18 hours. Only complete digestion of the fragment of tendon was accepted as evidence for the presence of a collagenase. We subsequently used dried collagen prepared by the method of Bergmann as modified by Einbinder and Schubert (32), and with this material it was possible to get accurate quantitative estimations of collagenolytic activity; for the preliminary survey we were interested primarily in the mere occurrence of col-

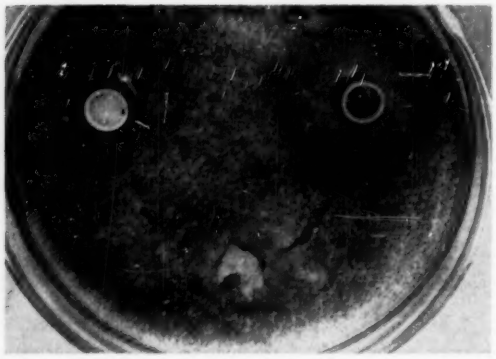


FIG. 1. AZOCOLL AGAR PLATE SHOWING DIGESTION OF THE AZOCOLL

lagenases, our only quantitative studies being obtained from the highest dilution of culture which gave complete digestion of the tendon.

b. *"Denatured" collagen:* For "denatured" collagen Azocoll was employed as a substrate. "Azocoll" is the name given by Oakley, Warrack, and Van Heyningen (21) to a preparation of hide powder coupled with a red azo-dye. Hydrolysis of the hide powder results in the liberation of the dye, permitting an estimation of enzymatic activity by colorimetric means (21, 22).

In the present study Azocoll has been used in several different ways.

A standard amount of culture filtrate has been added to a weighed sample of Azocoll, allowed to react under standard conditions, and the amount of color released estimated. This technique, which is essentially that of Oakley *et al.* (21), is peculiarly adapted for quantitative estimations and is described in detail elsewhere (33).

A simpler method for qualitative studies has been to disperse 70 to 100 mg. in 10 ml. of nutrient agar in a petri dish. The resulting agar plate appears diffusely red, not unlike a blood agar plate. When test organisms are streaked across such a plate and incubated for 12 to 18 hours at 37° C., the presence of an enzyme capable of digesting the Azocoll is indicated by the appearance of a clear zone similar to that seen around a hemolytic organism on a Blood Agar Plate (Figure 1).

With organisms such as *Proteus vulgaris* that give a spreading type of growth, another technique may be employed. The organisms are grown in a fluid medium and then the clarified culture medium is inoculated into small porcelain cups, placed on an Azocoll plate as in antibiotic testing. The plate is then incubated at 37° C. and examined periodically for signs of clarification of the Azocoll around the porcelain cups (Figure 1).

It may again be noted here that Azocoll is not a specific substrate for collagenases. Other proteolytic enzymes which have little or no action on collagen will attack it. On the other hand, the use of fresh unaltered collagen is a true indicator of collagenase activity.

RESULTS

In all, 162 strains of bacteria were examined by the methods detailed above. The results of the study are set out in the following tables (I and II).

These studies show that, apart from two species of anaerobic spore-bearing bacilli, *Cl. perfringens* and *Cl. histolyticum*, no organism examined was capable of digesting native collagen. The ability to produce enzymes active against Azocoll was rather commoner, being found in almost all clostridia in the series and in certain strains of pyococci, of aerobic spore-bearers (Bacilli) and of *P. vulgaris*. The enzymes, both collagenases and proteinases elaborated by *Cl. histolyticum* were by far the most potent of any studied.

With regard to the other organisms, the significance of our findings may be summarized as follows.

1. *Cocci:* Although none of the pyococci investigated produced a true collagenase, one out of six strains of *Staph. aureus* was capable of attacking Azocoll, although only weakly. The fact that even one strain of staphylococcus could hydrolyze Azocoll is of interest, not only because the proteolytic activities of this organism have been considered negligible, but also because Gillespie and Adler (34) in a recent study report that coagulase-positive staphylococci are without action on Azocoll. As for the hemolytic streptococci, our findings agree with those of Todd (28) and Elliott and Dole (35, 36). It is probable that the positive reactions obtained against Azocoll are due to the proteinase described by Elliott as active against streptococcal M-protein.

2. *Aerobic spore-bearers:* The ability of four out of the nine strains of *Bacillus* examined to pro-

TABLE I

Miscellaneous organisms (50 strains)

Organism	No. of strains	Collagen positive	Azocoll positive
Staph. aureus	6	0	1 (±)
Staph. albus	6	0	0
Strept. pyogenes	5	0	2 (±)
B. mesentericus	3	0	2
B. cereus	4	0	2
B. anthracis	2	0	0
E. coli	6	0	0
P. vulgaris	6	0	2
Myxobacteria	3	0	0

duce enzymes capable of degrading hide-powder was unexpected. While most members of this genus can liquefy gelatin, few can attack the higher proteins. It would probably be of interest to compare the activities of these proteinases against a wide variety of substrates. No strain produced a collagenase, not even *B. anthracis*, which gives rise to an extremely necrotic type of lesion in the animal body.

3. *Gram negative bacilli:* Among the strains of *Enterobacteriaceae* tested, the results were negative both with strains of *E. Coli* and *Proteus vulgaris* even though the latter species may cause massive destruction of tissue in some wound infection. Dr. Wm. Altemeier has stated in personal communications that strains of *Proteus vulgaris* exist which produce an active collagenase. However, these collagenases, unlike those elaborated by the Clostridia, appear to be adaptive enzymes, and our methods of examination were not well suited to the detection of such enzymes. Indeed, it is worth suggesting that further surveys of this group of bacteria be made in which collagen or gelatin is incorporated in the test medium.

4. *Myxobacteria:* The three strains of *Chondrococcus columnaris* were included because they can cause in fish a highly fatal infection associated with much destruction of tissue (37). None of the strains produced enzymes capable of attacking hide powder, although Ordal and Rucker (37) state that gelatin is rapidly liquefied.

5. *Clostridia:* Apart from the *Cl. perfringens* and *Cl. histolyticum*, no species of clostridium examined could liquefy collagen. The ability of *Cl. sporogenes* to attack Azocoll may be significant since it is an actively proteolytic organism which has been used in the past for the enzymic debridement of foul necrotic wounds (38, 39).

It has been known for some time that *Cl. perfringens* could produce a collagenase. The distribution of this enzyme amongst the various toxigenic types has been set out in Table III. This table is in general agreement with the findings of Oakley *et al.* (21) in that all Type A strains can digest collagen. However, Oakley and his collaborators (21) report that Types B, C, and D also elaborate collagenase, although only in small amounts. This we did not encounter. However, the number of strains we studied was relatively small. Furthermore all four Type B strains and one out of three Type C strains gave positive reactions against Azocoll. Since Type B strains form at least two proteinases (29), one capable of attacking "native" and "denatured" collagen, and the other only "denatured" collagen, while Type C strains form only the true collagenase, some of the positive Azocoll results may be due to traces of collagenase, too weak to digest the relatively large amounts of fresh tendon, but capable of releasing color from Azocoll powder.

TABLE II

Clostridia

Organism	No. of strains	Collagen positive	Azocoll positive
Cl. perfringens	30	20	25
Cl. histolyticum	82	82	82
Cl. novyi	2	0	0
Cl. septicum	2	0	1
Cl. bifermentans	2	0	2
Cl. sporogenes	3	0	3

TABLE III

Cl. perfringens

Organism	No. of strains	Collagen positive	Azocoll positive
Type A	20	20	20
B	4	0	4
C	3	0	1
D	3	0	0

Indeed, even with Type A strains the amounts of collagenase produced are small—the highest dilution of culture giving positive results being $1/16$, as compared with frequent readings of $1/256$ and occasionally of $1/512$ in the case of *Cl. histolyticum*.

Cl. histolyticum

In view of the relatively high activity of the proteolytic enzymes produced by *Cl. histolyticum*, we decided to limit our future investigations to this organism. Hence we re-examined our collection of 82 strains and selected two—No. H.4 for its ability to produce proteinase and No. 230–2 for its ability to produce collagenase. Strain No. H.4 was isolated in the United States during World War II in the course of the Contaminated Wounds Project, while Strain No. 230–2 came from the collection of Dr. L. S. McClung of Indiana University. Further details concerning these organisms are not available.

Development of optimal medium to produce enzymes

Since this study was undertaken in an effort to discover bacterial enzymes of therapeutic value, the development of a fluid medium which would consistently favor the production of large amounts of a highly active proteinase by *Cl. histolyticum* has involved an extensive series of trials. It soon became clear that one containing high concentrations of peptone was superior to all others. The effect of peptone concentration, vitamins, minerals, pH, ionic strength, and Fe^{++} was then studied, and the following formula devised.

To each liter of distilled water are added:

15	Gm.	Trypticase Soy Base
50	Gm.	Proteose Peptone
9	Gm.	Na_2HPO_4
1.92	Gm.	KH_2PO_4
0.08	Gm.	$MgSO_4 7H_2O$
5	ml.	Vitamin Solution containing 200 mg. each of Ca Pantothenate, Nicotinic Acid, Pyridoxine and Thiamine, 20 mg. of Riboflavin per liter
6	ml.	$FeSO_4 7H_2O$/100 ml. N/10 HCl NaOH to adjust pH to 7.4

The concentration of the Vitamin Solution does not appear to be of critical value; that of the ferrous salt and the peptone does. Moreover, certain other metallic salts—in particular calcium, magnesium and cobalt—may have a beneficial effect, either in place of or in addition to the ferrous iron.

The maximum yield of enzyme is obtained after from 17 to 18 hours' incubation at 37° C., the warm medium being heavily inoculated with a young culture of the selected strain of *Cl. histolyticum* (Figure 2).

Other bases and supplements tested, including various casein-digests, phytones and peptones, collagen and collagen derivatives such as hide powder and gelatin, glucose and other carbohydrates were not as satisfactory.

Although the above medium was best in our studies, it would be extremely expensive to use on a commercial scale. We have, accordingly, tested various digest preparations as an alternative source of protein. Through the courtesy of Dr. Irwin S. Danielson of Lederle Laboratories seven preparations were obtained and tested in various ways. These preparations are listed in Table IV.

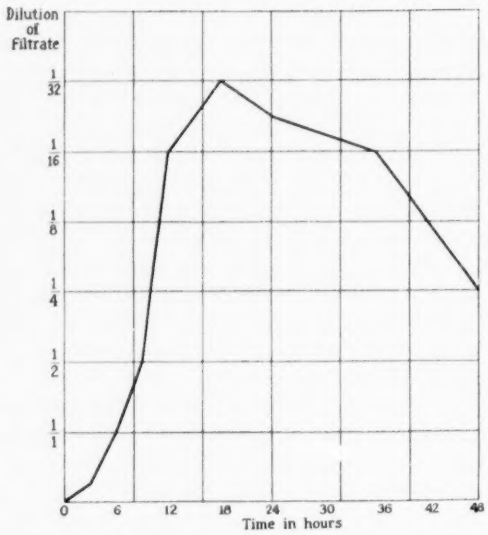


FIG. 2. COLLAGENASE PRODUCTION

Maximum collagenase activity is plotted in dilutions of the filtrate against time in hours.

None of these media, in varying concentrations and supplemented by various salts, vitamins and growth factors, has given us results in any way comparable to those obtained with our own medium. The most satisfactory was the Pancreatic Liver Cake Digest; and yet even at its best this gave us yields of the proteinase at least 50 per cent lower.

The very high (5 per cent) concentration of proteose peptone required for optimum yields suggested to us that some "growth factor" might be present. The peptone preparations used in bacteriological work contain considerable amounts of Blood Group A substance, but neither the addition of purified Blood Group A substance nor of various other fractions obtained from peptone in

TABLE IV

1. Pancreatic Beef Heart Digest
2. Pancreatic Liver Cake Digest
3. Peptic Liver Cake Digest
4. Peptic Veal Muscle Digest
5. Peptic Placenta Digest
6. Papain Liver Cake Digest
7. Tryptic Beef Heart Digest

any way enhanced the value of a medium in which proteose peptone was absent or present in sub-optimal amounts. Other attempts to separate a "growth factor" proved no more successful.

A technical point of importance is that the strains of *Cl. histolyticum* used in these studies have a tendency to develop rough variants. Such variants give consistently low yields of proteolytic enzymes. It has, therefore, been our custom periodically to plate out the strain under study on Blood Agar and to select a smooth colony from which to grow the inoculum in ordinary nutrient broth. By selecting several such colonies from a single plate and testing out the resultant broth cultures both for collagenase and proteinase activity, we have had no difficulty in maintaining strains of high potency.

SUMMARY AND CONCLUSIONS

1. Methods for determining a) the collagenase and b) other proteinase activities of micro-organisms have been described.

2. One hundred and sixty-two strains of bacteria have been examined for such activities.

3. Few organisms other than the spore-bearing bacilli (aerobic and anaerobic) exhibited proteinase activity, none other than the clostridia possessed collagenase activity.

4. By far the most active organism, both with regard to proteolytic and collagenolytic powers, was *Cl. histolyticum*.

5. A collection of 82 strains of this bacterium was examined in detail and certain very potent strains selected for further study.

6. A medium which favors greatly increased production of this proteinase has been evolved.

7. Unsuccessful attempts to simplify this medium are described.

REFERENCES

1. Bienstock, B., Ueber die Bakterien der Fäces. Ztschr. f. klin. Med., 1884, **8**, 1.
2. Lüderitz, C., Zur Kenntnis der Anaëroben Bakterien. Ztschr. Hyg. u. Infektionskrank, 1889, **5**, 141.
3. Sanfelice, F., Contributo alla morfologia e biologia dei batteri saprogeni aerobi ed anaerobi. Ann. d. Ist. d'ig., sper. d. Univ. di Roma, 1890, **2**, 99.
4. Bienstock, B., Recherches sur la putréfaction. Ann. Inst. Pasteur, 1900, **13**, 854.
5. Bienstock, B., Du rôle des bactéries de l'intestin. Ann. Inst. Pasteur, 1900, **14**, 750.
6. Klein, E., Ein Beitrag zur Bakteriologie der Leichenverwesung. Zentralbl. f. Bakt. (1 Abt.), 1899, **25**, 278.
7. Klein, E., Zur Kenntnis und Differentialdiagnose einiger Anaërobier. Zentralbl. f. Bakt. (1 Abt. Orig.), 1901, **29**, 991.
8. Tissier, H., and Martelly, Recherches sur la putréfaction de la viande de boucherie. Ann. Inst. Pasteur., 1902, **16**, 865.
9. Dernby, K. G., and Blanc, J., On the growth and the proteolytic enzymes of certain anaerobes. J. Bact., 1921, **6**, 419.
10. Kendall, A. I., Day, A. A., and Walker, A. W., Metabolism of *B. welchii, Vibrion septique, B. fallax, B. tertius, B. tetani, B. pseudotetani, B. botulinus, B. bifermentans, B. oedematiens, B. acrofœtidus, B. sporogenes, B. histolyticus* and *B. putrificus*. Studies in bacterial metabolism, XLIV–LV. J. Infect. Dis., 1922, **30**, 141.
11. Wolf, C. G. L., Contributions to the biochemistry of pathogenic anaerobes. VI. The proteolytic action of *Bacillus sporogenes* (Metchnikoff) and *Bacillus welchii*. J. Path. & Bact., 1918–19, **22**, 270.
12. Wolf, C. G. L., and Harris, J. E. G., Contributions to the biochemistry of pathogenic anaerobes. III. The effect of acids on the growth of *Bacillus welchii* (*B. perfringens*) and *Bacillus sporogenes* (Metchnikoff). Biochem. J., 1917, **11**, 213.
13. Wolf, C. G. L., and Harris, J. E. G., Contributions to the biochemistry of pathogenic anaerobes. IV. The biochemistry of *Bacillus histolyticus*. J. Path. & Bact., 1922, **22**, 1.
14. Henry, H., On *B. welchii* haemotoxin and its neutralisation with antitoxin. J. Path. & Bact., 1922, **25**, 1.
15. MacFarlane, R. G., and MacLennan, J. D., The toxemia of gas-gangrene. Lancet, 1945, **2**, 328.
16. Weinberg, M., and Randin, A., Ferment fibrolytique d'origine microbienne. Compt. rend. Soc. de biol., 1931, **107**, 27.
17. Weinberg, M., and Randin, A., Propriétés physico-chimiques du ferment fibrolytique d'origine microbienne. Compt. rend. Soc. de biol., 1932, **110**, 352.
18. Maschmann, E., Über Bakterienproteasen. II. Biochem. Ztschr., 1937–38, **295**, 1.
19. Maschmann, E, Über Bakterienproteasen. III. Mitteilung. Die Proteasen des *B. perfringens*. Biochem. Ztschr., 1937–38, **295**, 351; IV. Mitteilung. Die Proteasen des *B. histolyticus*. Biochem. Ztschr., 1937–38, **295**, 391.
20. Maschmann, E., Zur Kenntnis der Proteasen des *B. sporogenes*. Über eine neue Proteinase und über aktivierbare Peptidasen. Biochem. Ztschr., 1938, **300**, 89.
21. Oakley, C. L., Warrack, G. H., and Van Heyningen, W. E., The collagenase (κ toxin) of *Cl. welchii* Type A. J. Path. & Bact., 1946, **58**, 229.
22. Bidwell, E., and Van Heyningen, W. E., The biochemistry of the gas gangrene toxins. 5. The K-

toxin (collagenase) of *Clostridium welchii*. Biochem. J., 1948, **42**, 140.

23. Jennison, M. W., Bacterial collagenase. J. Bact., 1945, **50**, 369.
24. Jennison, M. W., The collagenase activity of culture filtrates of *Clostridium histolyticum*. J. Bact., 1947, **54**, 55.
25. Evans, D. G., The production of certain species of *Clostridium* of enzymes disintegrating hide powder. J. Gen. Microbiol., 1947, **1**, 378.
26. Evans, D. G., and Prophet, A. S., Enzymes of *Clostridium welchii* Type A and *Clostridium histolyticum* that disintegrate decalcified human tooth dentine. J. Gen. Microbiol., 1950, **4**, 360.
27. Evans, D. G., and Prophet, A. S., Disintegration of human dentine by bacterial enzymes. Lancet, 1950, **1**, 290.
28. Todd, E. W., A study of the inhibition of streptococcal proteinase by sera of normal and immune animals and of patients infected with group A hemolytic streptococci. J. Exper. Med., 1947, **85**, 591.
29. Oakley, C. L., Warrack, G. H., and Warren, M. E., The Kappa and Lambda antigens of *Clostridium welchii*. J. Path. & Bact., 1948, **60**, 495.
30. Bidwell, E., The α-toxin (collagenase) of *Clostridium welchii*. 2. The effect of alkaline pH and heat. Biochem. J., 1949, **44**, 28.
31. Bidwell, E., Proteolytic enzymes of *Clostridium welchii*. Biochem. J., 1950, **46**, 589.
32. Einbinder, J., and Schubert, M., Binding of mucopolysaccharides and dyes by collagen. J. Biol. Chem., 1951, **188**, 335.
33. Mandl, I., MacLennan, J. D., and Howes, E. L., Isolation and characterization of proteinase and collagenase from *Cl. histolyticum*. J. Clin. Invest., 1953, **32**, 1323.
34. Gillespie, W. A., and Adler, V. G., Production of opacity in egg-yolk media by coagulase-positive staphylococci. J. Path & Bact., 1952, **64**, 187.
35. Elliott, S. D., A proteolytic enzyme produced by group A streptococci with special reference to its effect on the type-specific M antigen. J. Exper. Med., 1945, **81**, 573.
36. Elliott, S. D., and Dole, V. P., An inactive precursor of streptococcal proteinase. J. Exper. Med., 1947, **85**, 305.
37. Ordal, E. J., and Rucker, R. R., Pathogenic myxobacteria. Proc. Soc. Exper. Biol. & Med., 1944, **56**, 15.
38. Donaldson, R., Character and properties of the "Reading" bacillus, on which a new method of treatment of wounds has been based. J. Path. & Bact., 1918–19, **22**, 129.
39. Donaldson, R., and Joyce, J. L., A method of wound treatment by the introduction of living cultures of a spore-bearing anaerobe of the proteolytic group. Lancet, 1917, **2**, 445.

ISOLATION AND CHARACTERIZATION OF PROTEINASE AND COLLAGENASE FROM CL. HISTOLYTICUM [1, 2]

By INES MANDL, JOHN D. MacLENNAN, and EDWARD L. HOWES with the technical assistance of ROBERT H. DeBELLIS and ARTHUR SOHLER

(From the Departments of Surgery and Microbiology, College of Physicians and Surgeons, Columbia University, New York, N. Y.)

(Submitted for publication February 20, 1953; accepted August 18, 1953)

INTRODUCTION

Reports on the nature of proteolytic enzymes obtained from *Cl. histolyticum* have been contradictory. Maschmann (1) described two extracellular and one intracellular enzymes besides peptidases. His extracellular proteinases are inhibited by cysteine, Fe^{++}, Cu, Hg and cysteine plus Fe^{++}. Weil and Kocholaty (2), on the other hand, found a proteinase activated by heavy metals and SH-compounds. Van Heyningen (3) tried to reconcile both findings by postulating two proteinases, one activated by cysteine, the other inhibited, so that the addition of cysteine to a system containing both enzymes would be without obvious effect. More recently, Kocholaty and Krejci (4) found that by salt precipitation they obtained one single proteinase only, activated by Fe^{++} and cysteine. Collagenase activity of *Cl. histolyticum* has been reported by Jennison (5), Evans (6), Oakley and Warrack (7), and Neumann and Tytell (8). Tytell and Hewson (9) prepared both a collagen-digesting enzyme inhibited by Fe^{++} and cysteine and a proteinase resembling that of Kocholaty and Krejci (4) and Van Heyningen (3). A similar proteinase has now been isolated by Lepow, Katz, Pensky, and Pillemer (10) except that their purified enzyme is optimally activated by cysteine alone, Fe^{++} being without effect.

Our own studies of *Cl. histolyticum* enzymes have led us to the conclusion that all strains elaborate a collagenase and at least one other proteolytic enzyme. The amounts vary with the strains and with the medium used. Elsewhere (11) the screening of organisms was described for the selection of strains capable of producing large amounts of potent enzymes that digested native and denatured collagen. By differential precipitation techniques we have now been able to isolate some of these enzymes from media growing certain of these strains.

Precipitation of proteinase

Optimum growth conditions for proteinase production are described in a previous paper (11). After 17 hours incubation the fluid medium containing the enzyme is freed of bacteria by passage through a large Seitz filter. It is then subjected to fractional precipitation under controlled conditions of temperature, pH and ionic strength. Methanol precipitation gives a more active and purer proteinase than precipitation with acetone, ethanol, propanol and butanol. Relatively high concentrations of 66 to 72 per cent methanol, pH of 6.1 to 6.2 and high ionic strength of 0.27 were found most satisfactory (see Tables I and II). At high ionic strength only traces of collagenase are co-precipitated.

The cooled filtrate adjusted to pH 6.1 is precipitated at $-5°$ C. with 2 to 2½ vol. methanol precooled to -15 to $-20°$ C. The precipitate is allowed to settle overnight at a temperature of $-15°$ C., then spun off in a refrigerated centrifuge. It is resuspended in a small amount of water, dialyzed, clarified by filtration or centrifugation and lyophilized to a white powder.

The small amount of collagenase co-precipitated under these conditions comes down with the first 1½ vol. methanol. When this fraction was centrifuged off and a further volume of methanol added to the supernatant, the second precipitate so obtained was a proteinase of high activity and completely free of collagenase (Fraction II). Although these enzyme preparations appear electrophoretically homogenous (Figure 1), they contain relatively large amounts of a saccharide im-

[1] This work supported in part by a grant from the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, Contract No. DA 49-007-md-14.

[2] Presented in part at the American Chemical Society Meeting, Atlantic City, Fall 1952.

TABLE I*

Precipitation of Cl. histolyticum proteinase under varying conditions

Vol. CH_3OH	pH	Azocoll test	Casein test (15)
½	7.5	no ppt	
¾	7.5	no ppt	
1	7.5	52	85
1½	7.5	70	90
2†	7.5	170	197
1½	5.5	19	84
1½†	6.0	60	180
1½	6.6	43	175
1½	7.0	38	139
1½	7.5	35	90
1½	8.4	25	90
2†	6.1	520	268
2	6.3	199	158
2	6.55	186	155
2	6.65	143	142
2	6.85	157	152

* Azocoll and Casein digestion values are given as comparative colorimetric readings per unit volume.
† Optimal conditions.

purity which was found to be Blood Group A substance, co-precipitated from the peptone in the medium. This anti-hemagglutination factor, which is present in relatively large amounts in most mammalian tissue digests, is not precipitated to the same extent by $(NH_4)_2SO_4$. The amount of contaminant was, therefore, substantially reduced by preliminary precipitation of both enzymes with $(NH_4)_2SO_4$. Forty per cent $(NH_4)_2SO_4$ precipitated collagenase containing 20 to 40 per cent proteinase activity (35 per cent by weight). This proteinase could be separated by methanol precipitation of the dialysed collagenase preparation at pH 7.5 in the presence of enough $MgSO_4$ to raise the ionic strength to 0.27. The resulting enzyme preparation was free of collagenase and very active. Experiments on the further purification of this proteinase both by chemical and physico-chemical means are in progress.

TABLE II*

Effect of ionic strength and ions present on enzyme activity of precipitate (pH 6.1, 2 vol. CH_3OH added)

Ions present	$MgSO_4$ added (mg.)	Ionic strength	Azocoll test	Casein test (15)	B.A.A. test (17)
Fe^{++}	0	0.1	166	238	77
Fe^{++}	32	0.112	106	197	92
Fe^{++}	160	0.16	169	252	206
Fe^{++}	320	0.22	206	286	280
Fe^{++}	0	0.1	709	208	210
Mn^{++}	0	0.1	690	185	240
Mg^{++}	0	0.1	520	171	160
Co^{++}	0	0.1	510	149	240

* Azocoll, Casein and Benzoyl Arginine Amide digestion values are given as comparative colorimetric readings per unit volume.

Precipitation of collagenase

Precipitation of the clear Seitz filtrate with solid $(NH_4)_2SO_4$ gives a very active enzyme preparation. Most of the activity is in a first fraction obtained by adding 400 Gm. solid $(NH_4)_2SO_4$ to each liter of filtrate, allowing it to stand at 4° C. overnight, filtering, resuspending the precipitate in a little water, dialysing, filtering off any insoluble residue and freeze drying. Further purification may be obtained by calcium phosphate adsorption and elution with 10 per cent $(NH_4)_2SO_4$ or by a second $(NH_4)_2SO_4$ precipitation at more alkaline pH, but in our hands these steps entail considerable losses. The dried enzyme is not free of proteinases.

An alternative method is CH_3OH precipitation of the dialyzed Seitz filtrate essentially as described by Tytell and Hewson (9).

Assay methods employed

Azocoll: Various modifications of the method of Oakley, Warrack, and Van Heyningen (12) estimating the release of color from Azocoll (hide powder coupled to an azo dye) have been used to determine total proteolytic activity at consecutive steps of purification.

For quantitative determinations 50 mg. Azocoll are suspended in 9.9 ml. Phosphate Buffer pH 7.4 containing 4 parts M/15 Na_2HPO_4, 1 part M/15 KH_2PO_4 and 5 parts 0.9 per cent NaCl. One-tenth ml. enzyme solution is added and the flasks shaken for exactly 15 minutes on a Fisher Gyrosolver shaking machine placed in an incu-

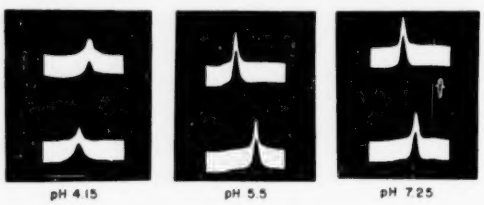


FIG. 1. ELECTROPHORETIC PATTERNS OF CL. HISTOLYTICUM PROTEINASE

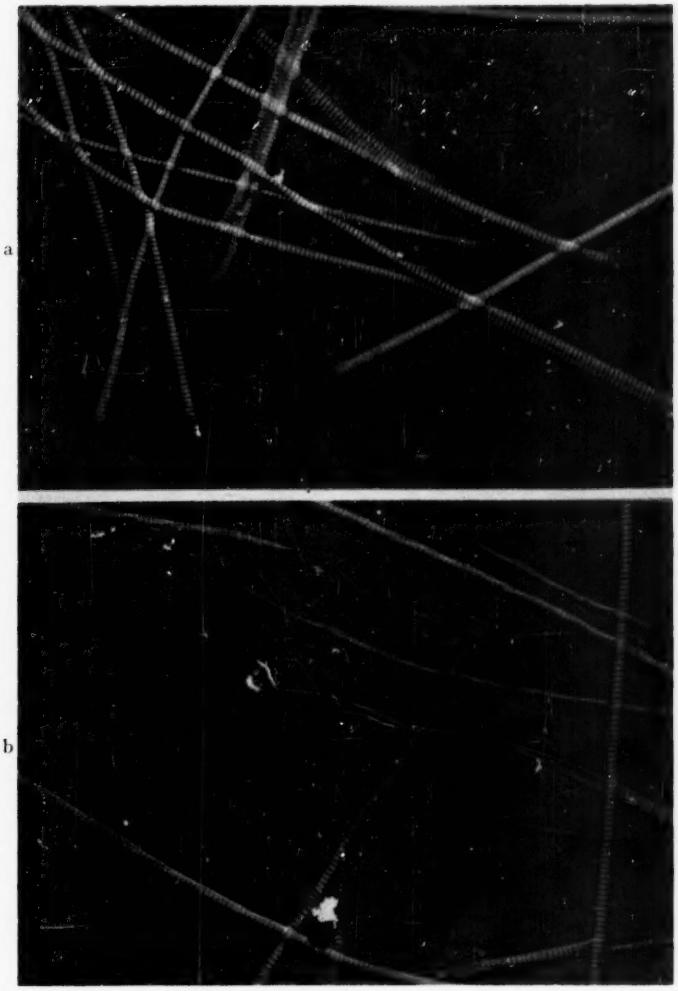


FIG. 2. ELECTRON MICROGRAPHS SHOWING EFFECT OF COLLAGENASE ON COLLAGEN FIBRILS. a. UNTREATED FIBRILS. b. COLLAGENASE TREATED FIBRILS
(Preparation by Dr. Jerome Gross, Mass. Inst. of Technology)

bator at 37° C. The Azocoll powder is then filtered off through a rapid filter paper directly into Klett tubes and the color released read in a colorimeter with a green filter No. 54. The Klett reading minus the blank is calculated for 1 mg. dry enzyme. This value indicates Q units for this enzyme preparation. When the effect of added activators, inhibitors, metal salts, etc. is tested the amount of buffer is decreased to give a total volume of 10 ml.

Native collagen: Unlike Azocoll or hide powder, which are attacked by collagenase and proteinase(s), "undenatured" collagen is a specific substrate for collagenase. Collagenase activity was therefore determined by the action of the enzyme on Achilles tendon or collagen purified by extraction with Na_2HPO_4 and KCl to remove soluble proteins and mucopolysaccharides (13). The degree of hydrolysis was established by a modification of the ninhydrin color reaction (14) for free amino groups and Kjeldahl nitrogen tests or by serial dilution experiments. The extent of the

breakdown of collagen by collagenase was estimated by occasional Van Slyke ninhydrin CO_2 tests (15) to distinguish between peptides and free amino acids. These tests showed that at least some amino acids were released from collagen, although enzymatic digestion was not as complete as acid hydrolysis. Paper chromatograms of enzymatic and acid collagen hydrolysates confirmed these findings. Since all our collagenase preparations contain some proteinase this enzyme may be responsible for the liberation of free amino acids after breakdown to the peptide level by the collagenase. We are still engaged in the elucidation of this point.

Dr. Jerome Gross of Massachusetts General Hospital has studied the changes in the fine structure of undenatured collagen fibrils as influenced by collagenase and proteinase under the electron microscope. The proteinase either with or without cysteine activation did not attack collagen fibrils. On the other hand, the attack brought about by collagenase was very striking. The fibrils became distinctly tapered and lost their cross striations. Trypsin fragments the fibril transversely but collagenase reduced the entire diameter as if it were washed away (Figure 2).

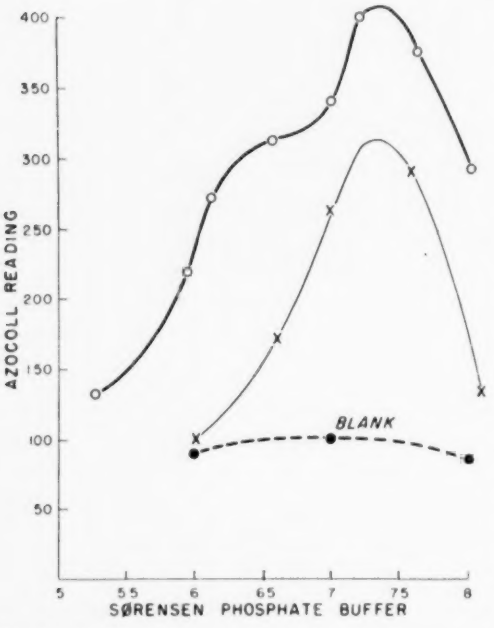


FIG. 3. ACTIVITY OF COLLAGENASE (o) AND PROTEINASE (x) IN SØRENSEN PHOSPHATE BUFFER

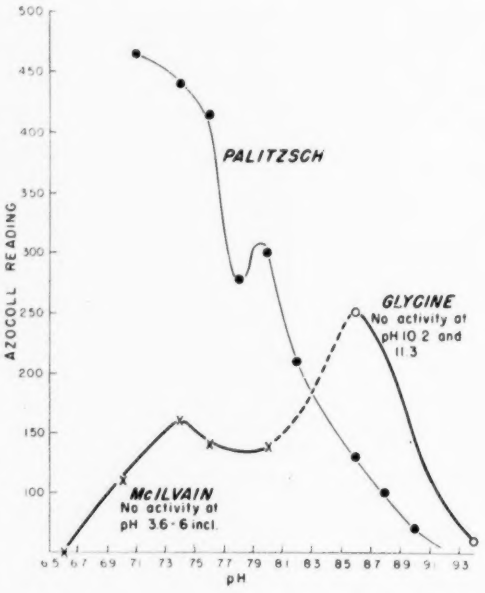


FIG. 4. ACTIVITY OF PROTEINASE IN PALITZSCH, McILVAIN, AND GLYCINE BUFFER

Other tissue components: None of our preparations has had any activity against elastin or keratin.

Other substrates: Enzymatic activity of our preparations was also measured against various other substrates such as casein, egg albumen, bovine serum albumen, hemoglobin, etc. by adaptation of Kunitz' method (16) of determining the tyrosine and tryptophane content of the supernatant obtained on trichloroacetic acid precipitation of the digest. For casein hydrolysis the decrease in turbidity resulting on addition of aliquots to sulfosalicylic acid at given intervals according to Grob (17) was also followed. In addition, synthetic substrates such as benzoyl arginine amide, benzoyl arginine isopropyl ester and arginine methyl ester were employed. No activity was found against tyrosine ester or glycine ester. Activity against benzoyl arginine amide was measured by Conway microdiffusion of the NH_3 released on splitting of the amide bond followed by nesslerization or titration by the method described for ficin (18). Hestrin's method (19) of reac-

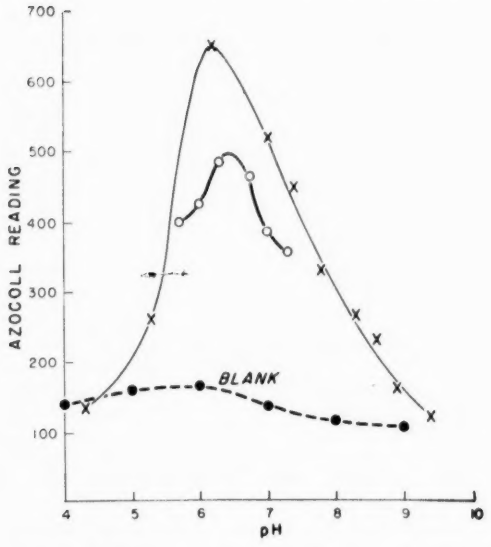


FIG. 5. ACTIVITY OF PROTEINASE IN UNBUFFERED SOLUTION AT DIFFERENT pH ESTABLISHED BY ADDING NaOH OR HCl.

tion with alkaline hydroxylamine was used to follow the decrease in unsplit ester present after enzymatic interaction.

Chemical properties of proteinase and collagenase

Both enzyme preparations are typical proteins, and give positive reactions with all the usual protein reagents. There is no evidence of free SH groups as judged by the nitroprusside reaction, although S is found on ashing. Their tyrosine and tryptophane content as determined by spectrophotometric analysis is low. These findings agree with those of Kocholaty and Krejci (4). The amino acid composition has been estimated by qualitative paper chromatography. It resembles that of collagen in both enzyme preparations. Quantitative analyses have been postponed until the purer enzymes are available.

In phosphate buffers both enzyme preparations showed maximum activity at pH 7.4 (Figures 3, 4) while with other buffers different maxima were obtained (Figure 4). Activities at a given pH also varied with the buffer employed. These differences are probably due to inhibition by some of the ions present—*e.g.*, borate and citrate (Figure 4).

The collagenase-free proteinase was more thoroughly investigated first. Hence, the rest of the discussion about the factors that influence activity will be largely about proteinase.

In McIlvain phosphate-citrate buffer no proteinase activity was discerned below pH 6.6 (Figure 4); in Sørensen phosphate buffer, while the maximum activity occurred at the same pH, the enzyme was active over a much wider range (Figure 3). An apparent second maximum in glycine buffer at pH 8.6 was probably caused by the difference in buffer composition (Figure 4). In Palitzsch borate buffer activity decreased with rising pH, with a possible slight maximum at pH 8 (Figure 4). When no buffer was used and pH adjusted with HCl and NaOH only, the maximum appeared at pH 6.4 (Figure 5). It is of interest in this connection that in 1934, Walbum and Reymann (20) found two maxima, one between pH 6 to 7 and one between pH 8 to 9, for optimum gelatinase activity of crude *Cl. histolyticum* filtrates. Since they too had to change the buffer systems from citrate (pH 4 to 5) to phosphate (pH 6 to 7) to borate (pH 8 to 10) the different effects of these anions may account for their results.

Stability at different pH values also differs with the buffer system employed. At low pH, stability is greater in acetate than in McIlvain buffer (Figure 6). For at least one month stability of dilute solutions is fair over a pH range from about 5.6

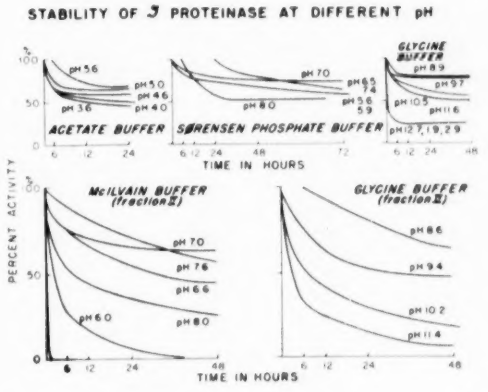


FIG. 6. STABILITY OF PROTEINASE AT DIFFERENT pH'S AND IN DIFFERENT BUFFERS

Fraction II is a purer preparation.

to high alkalinity, although stability is less for the more active highly purified preparations (Fraction II, Figure 6). In the dry state the lyophilized enzymes retain their activity indefinitely.

Table III shows the effect of various cations on proteinase activity. Hg^{++} is seen to inhibit the enzyme completely, Ag considerably, most metal ions reduce proteinase activity to some extent, but Ca^{++} increases it almost twofold; Mg, Co, and Mn activate slightly. Again, results differ somewhat with the system used, substrate and buffer (Table III).

The effects of oxidizing and reducing agents have been studied, but we were unable to confirm the cysteine activation or iodoacetamide inhibition claimed for *Cl. histolyticum* proteinase (*2*, 4, *7*, 9, 10), except for the splitting of the synthetic substrates. At various concentrations and under various conditions of incubation, essentially unchanged activity toward Azocoll and casein was obtained in the presence or absence of cysteine or iodoacetamide. Very high concentrations of cysteine (M/10) showed definite inhibition of all enzyme preparations. Cysteine plus a heavy metal did not change the effect of the metal alone, except in the case of Co where the slight increase of activity with Co alone was no longer observed. The lack of cysteine activation led to the belief that we may be dealing with an enzyme distinct from the cysteine activated proteinase described by other workers (*2*, 4, *7*, 9, 10).

We have now further evidence for this third enzyme which we propose to call δ enzyme. This

TABLE III

Effect of various cations on δ proteinase activity

Metal	Azocoll reading in McIlvain buffer	Azocoll reading in Ca borate buffer	Undigested casein after 1 hr.	Undigested casein after 3 hrs.
			%	%
Ca	540	—	18	0
Mg	420	284	20	0
Zn	435	157	100	62
Mn	415	240	25	0
Fe^{++}	390	248	28	0
Co	355	207	28	0
Cu	340	23 (Blank)	100	85
Fe^{+++}	296	158	100	50
Pb	250	131	100	40
Ni	210	24 (Blank)	100	100
Ag	112 (Blank)	41 (Blank)	100	100
Hg	100 (Blank)	122 ?	100	100
None (control)	360	157	25	0
Blank	150		100	100

TABLE IV

*Titration of proteinase against Azocoll using different antisera**

Serum	Anti-β	Anti-γ	Anti-δ
2001	100	100	100
2005	350	160	70
2008	370	360	170
2035	230	150	170
8003	260	550	110
8006	180	410	110

* The discrepancies between the anti-γ and anti-δ readings will be obvious and indicate that these two proteinases are antigenically distinct. (Sera were kindly supplied by Dr. C. L. Oakley who calibrated them and who has largely confirmed our results.)

evidence, which will be given in greater detail elsewhere, can be summarized as follows: Tests conducted with a series of six specific antisera calibrated by Oakley and Warrack (*7*) for anti β (collagenase) and anti γ (cysteine activated proteinase) activity show that the new δ proteinase is immunologically distinct from both β and γ (see Table IV).

Heating of the proteinase preparation to 50° for 30 minutes, lowering of the pH to 4.6, or passage through a column of the ion exchange resin XE64 (Roehm and Haas) results in a loss of ⅔ or more of the activity towards the synthetic substrates benzoyl arginine amide and arginine ester. This differential inactivation indicates the presence of at least two enzymes in the proteinase preparation. While collagenase is equally thermolabile and sensitive to lowering of pH, losses in activity of proteinase preparations are too great to be accounted for by traces of collagenase present in some of our preparations and inactivation is the same when collagenase-free preparations are subjected to heat and low pH.

We therefore found that at least three proteolytic enzymes are elaborated by *Cl. histolyticum:* a collagenase identical with Oakley's β enzyme, a cysteine activated proteinase, possibly Oakley's γ enzyme, that attacks certain synthetic substrates and resists heat and low pH values and a new more labile proteinase, unaffected by cysteine and similar reducing agents.

SUMMARY

Conditions for the separation of proteolytic enzymes from *Cl. histolyticum* have been established. A proteinase free of collagenase activity and a

powerful collagenase, which, although it still contains other proteinases, is considerably enriched and concentrated with regard to collagen digesting capacity have been isolated. The proteinase preparation has been investigated for the effect of pH, cations and anions on its activity. Its stability has been determined. The main constituent of the proteinase preparation made by the method described is believed to be a new enzyme.

ACKNOWLEDGMENTS

We wish to thank Dr. C. L. Oakley of the University of Leeds, England, for calibrated antisera and a generous supply of Azocoll.

We are further indebted to Dr. Jerome Gross, Massachusetts General Hospital for the electronmicrophotographs and permission to publish these before his own work is ready for press.

Dr. Harry Goldenberg of the Brooklyn Jewish Hospital very kindly put at our disposal the synthetic substrates benzoyl arginine isopropyl ester, arginine methyl ester, tyrosine ethyl ester and glycine ethyl ester, prepared by him.

The electrophoresis runs and photographs (Figure 1) were made by Dr. Daniel Moore of this institution.

REFERENCES

1. Maschmann, E., Über Bakterienproteasen. IV. Mitteilung. Die Proteasen des *B. Histolyticus*. Biochem. Ztschr., 1937–38, **295**, 391–399; see also IX. Mitteilung. Die Anaerobiase des Gasbranderregers. Biochem. Ztschr., 1938, **297**, 284.
2. Weil, L., and Kocholaty, W., Studies on the proteinase of *Clostridium histolyticum*. Biochem. J., 1937, **31**, 1255; see also Kocholaty, W., Weil, L., and Smith, L., Proteinase secretion and growth of *Clostridium histolyticum*. Biochem. J., 1938, **32**, 1685.
3. Van Heyningen, W. E., The proteinases of *Clostridium histolyticum*. Biochem. J., 1940, **34**, 1540.
4. Kocholaty, W., and Krejci, L. E., The activation mechanism and physico chemical properties of *Clostridium histolyticum* proteinase. Arch. Biochem., 1948, **18**, 1.
5. Jennison, M. W., Bacterial collagenase. J. Bact., 1945, **50**, 369; see also The collagenase activity of culture filtrates of *Clostridium hystolyticum*. J. Bact., 1947, **54**, 55.
6. Evans, D. G., The production by certain species of *Clostridium* of enzymes disintegrating hide powder. J. Gen. Microbiol., 1947, **1**, 378.
7. Oakley, C. L., and Warrack, G. H., The alpha, beta and gamma antigens of *Clostridium histolyticum* (Weinberg & Séguin, 1916). J. Gen. Microbiol., 1950, **4**, 365.
8. Neumann, R. E., and Tytell, A. A., Action of proteolytic enzymes on collagen. Proc. Soc. Exper. Biol. & Med., 1950, **73**, 409.
9. Tytell, A. A., and Hewson, K., Production, purification, and some properties of *Clostridium histolyticum* collagenase. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 555.
10. Lepow, I. H., Katz, S., Pensky, J., and Pillemer, L., A cysteine-activated proteinase of *Clostridium histolyticum*. J. Immunol., 1952, **69**, 435.
11. MacLennan, J. D., Mandl, I., Howes, E. L., Bacterial digestion of collagen. J. Clin. Invest., 1953, **32**, 1317.
12. Oakley, C. L., Warrack, G. H., and Van Heyningen, W. E., The collagenase (κ toxin) of *Cl. welchii* type A. J. Path. & Bact., 1946, **58**, 229.
13. Einbinder, J., and Schubert, M., Binding of mucopolysaccharides and dyes by collagen. J. Biol. Chem., 1951, **188**, 335.
14. Harding, V. J., and MacLean, R. M., A colorimetric method for the estimation of amino-acid α-nitrogen. J. Biol. Chem., 1915, **20**, 217; see also Harding, V. J., and Warneford, F. H. S., The ninhydrin reaction with amino-acids and ammonium salts. J. Biol. Chem., 1916, **25**, 319.
15. Van Slyke, D. D., MacFadyen, D. A., and Hamilton, P., Determination of free amino acids by titration of the carbon dioxide formed in the reaction with ninhydrin. J. Biol. Chem., 1941, **141**, 671.
16. Kunitz, M., Crystalline soybean trypsin inhibitor. II. General properties. J. Gen. Physiol., 1947, **30**, 291; see also Northrop, J. H., Kunitz, M., Herriott, R. M., Crystalline Enzymes, ed. 2, rev. and enl., New York, Columbia Univ. Press, 1948, p. 308.
17. Grob, D., Proteolytic enzymes. I. The control of their activity. J. Gen. Physiol., 1946, **29**, 219.
18. Mandl, I., and McLaren, A. D., The photochemistry of proteins. VII. A quantum yield for the inactivation of ficin by ultraviolet light. Arch. Biochem., 1949, **21**, 408.
19. Hestrin, S., The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and its analytical application. J. Biol. Chem., 1949, **180**, 249.
20. Walbum, L. E., and Reymann, G. C., Investigations into the nature of the gelatin-melting enzymes formed by the gas-gangrene bacteria; the importance of the degree of acidity of the medium for the action of the enzymes. J. Path. & Bact., 1934, **39**, 669.

AUTHOR INDEX

(Page numbers in italics refer to abstracts)

A

Abelmann, Walter H., and **Kowalski, Henry J.** The cardiac output in cirrhosis of the liver, at rest and on exercise *550*

—. See KOWALSKI and ABELMANN 1025

Abbot, Lynn D., Jr. See JAMES, ABBOT, NORBERG, BIRKELAND, and EVANS *578*

Adams, Margaret A. See LEVENSON, ADAMS, ROSEN, and TAYLOR 497

Adams, Raymond D. See MCDERMOTT and ADAMS *587*

Adams, William S., and **Figueroa, William G.** The ability of the human subject to regenerate plasma proteins during prolonged plasmapheresis *550*

Adlersberg, David, Wang, Chun-I, and **Schaefer, Louis E.** Extreme elevation of plasma lipids in the cholesterol-fed rabbit treated with cortisone and hydrocortisone *550*

Aikawa, Jerry K., Felts, John H., Jr., and **Harrell, George T., Jr.** Isotopic studies of potassium metabolism in diabetes 15

Albert A. See KEATING and ALBERT *580*

Albright, Edwin C., Larson, Frank C., and **Deiss, William P.** Iodine-containing amino acids in plasma: Failure to demonstrate 3:5:3′-L-Tri-iodothyronine *551*

Alexander, Benjamin, and **Goldstein, Robert.** Dual hemostatic defect in pseudohemophilia *551*

Almy, T. P. See SLEISENGER, ALMY, GILDER, and PERLE 466

Alper, B. J. See LIPSKY, ALPER, RUBINI, GORDON, and VAN ECK *584*

Altrock, Joan R. See BONDY and ALTROCK 703

Amatuzio, Donald S., Stutzman, Francis L., Vanderbilt, Muriel J., and Nesbitt, Samuel. Interpretation of the rapid intravenous glucose tolerance test in normal individuals and in mild diabetes mellitus 428

Ames, William H. See BEDELL, CULBERTSON, EHRENHAFT, AMES, and THEILEN *554*

Anderson, Arthur R. See FLINK, STUTZMAN, ANDERSON, and KONIG *568*

Anderson, Leighton L., Willcox, Mary L., Silliman, Joan, and **Blount, S. Gilbert, Jr.** The pulmonary physiology of normal individuals living at an altitude of one mile 490

Angrist, A. See LORANT, LORANT, ANGRIST, and KORPMAN 1005

Appelbaum, Emanuel. See FALK and APPELBAUM 39

Arends, Tulio. See RUNDLES, ARENDS, and COONRAD *690*

Armstrong, S. H., Jr., McLeod, Katherine, Wolter, Janet, and **Kukral, John.** The comparison of albumin and gamma globulin turnover times in the normal and severe cardiac failure of varied cause *552*

Asper, Samuel P., Jr., Selenkow, Herbert A., and **Plamondon, Charles A.** The metabolic activity of 3,5,3′-*l*-Triiodothyronine in myxedema *552*

Assali, N. S., Kaplan, S. A., Fomon, S. J., and **Douglass, R. A., Jr.** Renal function studies in toxemia of pregnancy. Excretion of solutes and renal hemodynamics during osmotic diuresis in hydropenia 44

Assali, N. S., Kaplan, S., Oighenstein, S., and **Suyemoto, R.** Hemodynamic effects of 1-hydrazinophthalazine (apresoline) in human pregnancy: Results of intravenous administration 922

Athens, John W. See FREINKEL, SCHREINER, and ATHENS 138

Atlas, Leon T. Minor respiratory diseases: Studies with four agents in human volunteers *552*

B

Balboni, F. See BING, SIEGEL, VITALE, BALBONI, and SPARKS *556*

Balls, Kent. See SOKOLOFF, WECHSLER, MANGOLD, BALLS, and KETY *202*

Bardawill, C. J., Britton, A., and **Wightman, K. J. R.** Phosphorus turnover in normal and leukemic leukocytes *553*

Barker, E. S. See SINGER, ELKINTON, BARKER, and CLARK *604*

Bartter, Frederic C. See HENNEMAN, BARTTER, FORBES, and DEMPSEY *574*

—. See LEAF, BARTTER, SANTOS, and WRONG *582*

—. See LEAF, BARTTER, SANTOS, and WRONG 868

Bass, David E. See KLEEMAN, BASS, and QUINN 736

Batterman, Robert C., and **Traeger, Cornelius H.** Steroid induced gout in rheumatoid patients with prolonged cortisone therapy *553*

Bauer, F. K., Blahd, W. H., and **Fields, M.** Ascitic fluid and plasma protein exchange. Studies with radioiodinated human serum albumin *553*

Bauer, Walter. See CLARK, BLAIS, and BAUER 769

Baum, G. See SCHEINBERG, BLACKBURN, SASLAW, RICH, and BAUM 720

Beard, Marion. See PITNEY, BEARD, and SANNEMAN *595*

Becker, Caroline. See MARTIN, MCKINNEY, GREEN, and BECKER 1171

Becker, David V., Rall, J. E., Peacock, Wendell, and **Rawson, Rulon W.** The effect of a thyrotrophic hormone preparation on the metabolism of radioiodine in euthyroid, hyperthyroid and acromegalic individuals 149

—. See RALL, ROBBINS, BECKER, and RAWSON *596*

Becker, William H. See FARBER, BECKER, and EICHNA 1145

Bedell, George N., Culbertson, James W., Ehrenhaft, Johann L., Ames, William H., and **Theilen, Ernest O.** Value of pulmonary "capillary" and left atrial pressure pulse waves in assessing the degree of mitral regurgitation in patients with mitral stenosis *554*

Belman, Sidney. See EISEN, BELMAN, and CARSTEN *564*

Benedict, Jean D., Yü, T. F., Bien, Edward J., Gutman, Alexander B., and **Stetten, DeWitt, Jr.** A further study of the utilization of dietary glycine nitrogen for uric acid synthesis in gout 775

—. See BIEN, YÜ, BENEDICT, GUTMAN, and STETTEN 778

Bennett, Leslie L. See LIDDLE, BENNETT, and FORSHAM 1197

Berdasco, Gloria A. See WESTON, HANENSON, GROSSMAN, BERDASCO, and WOLFMAN *611*

Berkman, James, Rifkin, Harold, and **Ross, George.** The serum polysaccharides in diabetic patients with and without degenerative vascular disease 415

Berlin, Nathaniel I., Tolbert, Bert M., and **Lee, Helen C.** The metabolism of glycine-2-C^{14} in man III. The urinary excretion of C^{14} and cumulative radiation dosimetry 1

—. The metabolism of glycine-2-C^{14} in man: Hippuric acid synthesis *554*

Berliner, Robert W. See ORLOFF, KENNEDY, and BERLINER 538

Bernstein, Jack. See PRINZMETAL, KENNAMER, SHAW, MAXWELL, and BERNSTEIN *595*

Bernstein, Stanley H., Weston, Raymond E., Ross, George, Grossman, Jacob, Hanenson, Irwin B., and **Leiter, Louis.** Studies on intravenous water diuresis and nicotine and pitressin antidiuresis in normal subjects and patients with liver disease 422

Berson, Solomon A., Yalow, Rosalyn S., Post, Joseph, Wisham, Lawrence H., Newerly, Katharina N., Villazon, Manuel J., and **Vazquez, Oscar N.** Distribution and fate of intravenously administered modified human globin and its effect on blood volume. Studies utilizing I^{131} tagged globin *22*

—, —, **Schreiber, Sidney S.,** and **Post, Joseph.** The distribution and fate of intravenously administered I^{131} labeled human serum albumin *554*

—, —, —, —. Tracer experiments with I^{131} labeled human serum albumin: distribution and degradation studies 746

Best, Maurice M., Wathen, Joan D., and **Brodsky, William A.** Relation of changes in colloidal osmotic pressure to body weight in patients with hepatic cirrhosis with ascites *555*

Biehl, J. Park. See HAMBURGER and BIEHL 391

Bien, Edward J., Yü, T. F., Benedict, Jean D., Gutman, Alexander B., and **Stetten, DeWitt, Jr.** The relation of dietary nitrogen consumption to the rate of uric acid synthesis in normal and gouty man 778

—. See BENEDICT, YÜ, BIEN, GUTMAN, and STETTEN 775

Bien, Edward. See ZIFF, KANTOR, BIEN, and SMITH 1253

Bierly, James N. See HAVENS, DICKENSHEETS, BIERLY, and EBERHARD *573*

Bierman, Howard R., Cordes, Fauno L., Kelly, Keith H., Littman, Aline M., Steinfeld, Jesse L., and **Yamauchi, Paul K.** The behavior of leukocytes in man following the introduction of heterologous blood group substances *555*

—, **Byron, R. L., Jr., Kelly, K. H., Gilfillan, R. S., White, L. P., Freeman, N. E.,** and **Petrakis, N. L.** The characteristics of thoracic duct lymph in man 637

Bigelow, Frederick S. Measurements of platelet-derived serum vasoconstrictor (serotonin) in normal subjects and in patients with hemorrhagic disease *555*

Billing, Barbara H., Conlon, Harold J., Hein, David E., and **Schiff, Leon.** The value of needle biopsy in the chemical estimation of liver lipids in man 214

Bing, R. J., Siegel, A., Vitale, A., Balboni, F., and **Sparks, E.** Metabolism of the human heart in vivo *556*

Binion, John. See TOBIAN and BINION *608*

Birchard, William H., and **Strauss, Maurice B.** Factors influencing the diuretic response of seated subjects to the ingestion of isotonic saline solution 807

Birkeland, Steffen. See JAMES, ABBOT, NORBERG, BIRKELAND, and EVANS *578*

Blackburn, I. See SCHEINBERG, BLACKBURN, and RICH 125

—. See SCHEINBERG, BLACKBURN, SASLAW, RICH, and BAUM 720

Blahd, W. H. See BAUER, BLAHD, and FIELDS *553*

Blais, Joseph A. See CLARK, BLAIS, and BAUER 769

Blakemore, A. H. See BRADLEY, SMYTHE, FITZPATRICK, and BLAKEMORE 526

Bliss, Eugene L., Sandberg, Avery A., Nelson, Don H., and **Eik-Nes, Kris.** The normal levels of 17-hydroxycorticosteroids in the peripheral blood of man 818

Blount, S. Gilbert, Jr. See ANDERSON, WILCOX, SILLIMAN, and BLOUNT 490

Bondy, Philip K., and **Altrock, Joan R.** Estimation of the rate of release of adrenal 17-hydroxycorticosteroids in the human being by the venous catheter technique, with a method for determining plasma 17-hydroxycorticosteroids 703

Bonner, C. D. See FISHMAN, DART, BONNER, LEADBETTER, LERNER, and HOMBURGER 1034

Bookman, John J. See DOLGER, KUPFER, BOOKMAN, and CARR *562*

Bortin, Leonard. See SHENKIN, NOVACK, GOLUBOFF, SOFFE, and BORTIN 459

—. See NOVACK, SHENKIN, BORTIN, GOLUBOFF, and SOFFE 696

Bourneuf, Jeanne M. See RUTSTEIN, INGENITO, and BOURNEUF *690*

Bradbury, James T. See HODGES, PAUL, ROUTH, DAUM, and BRADBURY *575*

Bradley, Stanley E., Smythe, C. M., Fitzpatrick, H. F., and **Blakemore, A. H.** The effect of a portacaval shunt on estimated hepatic blood flow and oxygen uptake in cirrhosis 526

—. See NICKEL, LOWRANCE, LEIFER, and BRADLEY 68

Briller, Stanley A. See KOSSMANN, BRILLER, and MARCHAND *581*

Britton, A. See BARDAWILL, BRITTON, and WIGHTMAN *553*

Brock, Loring L., and **Siegel, Alan C.** Studies on the prevention of rheumatic fever: The effect of time of initiation of treatment of streptococcal infections on the immune response of the host 630

Brodsky, William A., Rehm, Warren S., and **McIntosh, B. J.** Osmotic equilibria of tissues *556*

—. See BEST, WATHEN, and BRODSKY *555*

Brooks, Laura. See INKLEY, KRIEGER, and BROOKS *577*

—. See WILSON, BROOKS, and MOORE *612*

Brunschwig, Alexander. See HELLMAN, ROSENFELD, FUKUSHIMA, EIDINOFF, BRUNSCHWIG, and GALLAGHER *573*

Brust, Albert A., Hagans, James A., and **Reiser, Morton F.** The four year course of 103 patients with benign essential hypertension and the influence of doctor-patient relationship upon it *556*

Bruton, Ogden. See MOTULSKY and BRUTON *591*

Bucht, Härje. See JOSEPHSON, EK, BUCHT, WERKÖ, KALLAS, and GRIEG 1184

Bullock, Jane A. See SHETLAR, PAYNE, BULLOCK, PATRICK, HELLBAUM, and ISHMAEL 1208

Bunim, Joseph J. See DEANE, ZIFF, and BUNIM 879

Bunn, Paul A. Use of magnamycin and erythromycin in the treatment of pneumococcal pneumonia *557*

Burchenal, Joseph H., Murphy, M. Lois, Ellison, Rose Ruth, and **Karnofsky, David A.** Clinical evaluation of a new antimetabolite in the treatment of leukemia *557*

Burdick, Mary P. See ROGERS, BURDICK, and BURNETT *598*

Burnett, Charles H. See WILKINS, TINSLEY, CULBERTSON, BURROWS, JUDSON, and BURNETT 1101

Burnett, George R. See ROGERS, BURDICK, and BURNETT *598*

Burnum, J. F., Hickam, J. B., and **McIntosh, H. D.** The influence of hypocapnia on the circulation: Clinical significance in orthostatic hypotension *558*

—. See MCINTOSH, BURNUM, HICKAM, and WARREN *588*

Burns, Thomas W., Engel, Frank L., Viau, Alberto, Scott, James L., Jr., Hollingsworth, Dorothy R., and **Werk, Emile.** Studies on the interdependent effects of stress and the adrenal cortex on carbohydrate metabolism in man 781

Burrows, Belton. The relationship of thyroid hormone utilization to its serum level *558*

Burrows, Belton A. See WILKINS, TINSLEY, CULBERTSON, BURROWS, JUDSON, and BURNETT 1101

Butt, Hugh R. See FLEISHER and BUTT 674

Butterworth, C. E., Jr. The production of L. E. cells in normal individuals *558*

Byron, R. L., Jr. See BIERMAN, BYRON, KELLY, GILFILLAN, WHITE, FREEMAN, and PETRAKIS 637

Byers, Sanford O. See ROSENMAN, FRIEDMAN, and BYERS 121

C

Caraway, Wendell T. See SALTZMAN and CARAWAY 711

Cardon, Philippe V., Jr., and **Lukas, Daniel S.** Changes in cardiac output during the cold pressor test: Comparison of low-frequency ballistocardiograph and direct Fick methods *559*

Carr, Julius. See DOLGER, KUPFER, BOOKMAN, and CARR *562*

Carrerea, Ana, Reid, Virginia, and **Kurnick, N. B.** Differences in susceptibility of polymorphonuclear leucocytes from several species to alteration by systemic lupus erythematosus serum *559*

Carroll, Douglas, Cohn, Jerome E., and **Riley, Richard L.** Pulmonary function in mitral valvular disease: Distribution and diffusion characteristics in resting patients 510

Carsten, Mary E. See EISEN, BELMAN, and CARSTEN *564*

Cartwright, G. E. See LAHEY, GUBLER, CARTWRIGHT, and WINTROBE *322*

—. See LAHEY, GUBLER, CARTWRIGHT, and WINTROBE *329*

—. See GUBLER, LAHEY, CARTWRIGHT, and WINTROBE 405

Cassels, Donald E., and **Morse, Minerva.** The arterial blood gases, the oxygen dissociation curve, and the acid-base balance in polycythemia vera 52

—, —. Arterial blood gases and acid-base balance in normal children 824

—. See MORSE and CASSELS 837

Cathcart, Richard T., Field, William W., and **Richards, Dickinson W., Jr.** Comparison of cardiac output determined by the ballistocardiograph (Nickerson apparatus) and by the direct Fick method 5

Chalmers, Theodore N. See HILLS, CHALMERS, and WEBSTER *574*

Chalmers, Thomas C., Eckhardt, Richard D., Reynolds, William E., Cigarroa, Joaquin G., Deane, Norman, Reifenstein, Robert W., and **Smith, Clifford W.** The relative effects of strict bed rest and dietary components in the treatment of acute infectious hepatitis *559*

Chalmers, T. M. See HILLS, CHALMERS, WEBSTER, and ROSENTHAL 1236

Chambers, Robert. See KUNA and CHAMBERS 436

Chaplin, H., Jr., Mollison, P. L., and **Vetter, H.** The body/venous hematocrit ratio: Its constancy over a wide hematocrit range 1309

Chapman, Don W., and **Livesay, W. R.** Cardiovascular effects of aramine *560*

Chart, J. J., and **Shipley, E. S.** The mechanism of sodium retention in cirrhosis of the liver *560*

Cherniack, R. M. The effect of mechanical exsufflation on respiratory gas exchange in chronic pulmonary emphysema 1192

Chodos, Robert B., and **Moses, John.** An evaluation of the pathologic physiology of the anemia of cancer *560*

Cigarroa, Joaquin G. See CHALMERS, ECKHARDT, REYNOLDS, CIGARROA, DEANE, REIFENSTEIN, and SMITH *559*

Clark, J. K. See SINGER, ELKINTON, BARKER, and CLARK *604*

Clark, William S., Blais, Joseph A., and **Bauer, Walter.** Observations concerning the clinical and metabolic effects of corticosterone (compound B) in rheumatoid arthritis 769

Cline, Frank, Jr. See MARTIN, CLINE, and MARSHALL 617

Cohn, Jerome E. See CARROLL, COHN, and RILEY 510

Coleman, Daniel H. See FINCH and COLEMAN *567*

Colville, Virginia F. See ZARAFONETIS and COLVILLE *614*

Conlon, Harold J. See BILLING, CONLON, HEIN, and SCHIFF 214

Conn, Hadley L., Jr., Wood, John C., and **Schmidt, Carl F.** A comparison of renal blood flow results obtained in the intact animal by the nitrous oxide (derived Fick) method and by the para-amino-hippurate (direct Fick) method 1180

Coonrad, Evelyn V. See RUNDLES, ARENDS, and COONRAD *600*

Corcoran, A. C. See DUSTAN, MASON, and CORCORAN *60*

Cordes, Fauno L. See BIERMAN, CORDES, KELLY, LITTMAN, STEINFELD, and YAMAUCHI *555*

Cotzias, George C. See DOLE, DAHL, SCHWARTZ, COTZIAS, THAYSEN, and HARRIS 185

Cournand, Andre. See FERRER, HARVEY, SAMET, and COURNAND *566*

Craig, James W. See MILLER, CRAIG, and WOODWARD *591*

Cranley, J. J. See CURRENS, KHAMBATTA, CRANLEY, and GORDON *561*

Crosley, Archer, P., Jr., Rowe, George G., McKenna, Roger, and **Crumpton, Charles W.** The metabolic and hemodynamic response of the human hypertensive kidney to a standard intravenous dose of 1-hydrazinophthalazine (apresoline) *561*

Crumpton, Charles W. See CROSLEY, ROWE, MCKENNA, and CRUMPTON *561*

Culbertson, James W., Easton, James G., and **Welton, Ted L.** Evidence for hepatocellular dysfunction in patients with arterial hypertension without signs of intrinsic hepatic or renal disease or congestive myocardial decompensation *561*
—. See BEDELL, CULBERTSON, EHRENHAFT, AMES, and THEILEN *554*
—. See WILKINS, TINSLEY, CULBERTSON, BURROWS, JUDSON, and BURNETT. 1101
Currens, J. H., Khambatta, R. B., Cranley, J. J., and **Gordon, Ida.** The effect of intravenous protoveratrine on digital pulse-volume and skin temperature *561*

D

D'Agostino, Angelo, Leadbetter, Wyland F., and **Schwartz, William B.** Alterations in the ionic composition of isotonic saline solution instilled into the colon 444
Dahl, Lewis K. See DOLE, DAHL, SCHWARTZ, COTZIAS, THAYSEN, and HARRIS 185
Daily, William M., and **Sheffield, Roy S.** Orthostatic factors in pulsus alternans *562*
Danowski, T. S. See GREENMAN, WEIGAND, and DANOWSKI *572*
Darragh, J. H., Womersley, R. A., and **Meroney, W. H.** Fructose in the treatment of diabetic ketosis *562*
—, —, —. Fructose in the treatment of diabetic ketosis 1214
Dart, R. M. See FISHMAN, DART, BONNER, LEADBETTER, LERNER, and HOMBURGER 1034
Daughaday, William H. See ROSECAN and DAUGHADAY *599*
Daum, Kate. See HODGES, PAUL, ROUTH, DAUM, and BRADBURY *575*
Davidson, Charles S. See SCHWARTZ, SEEGMILLER, PHILLIPS, GABUZDA, and DAVIDSON *602*
—. See GABUZDA, PHILLIPS, and DAVIDSON 899
Davis, Robert K. See ROSENBAUM, NELSON, STRAUSS, DAVIS, and ROSSMEISL 394
Deane, Norman, Ziff, Morris, and **Bunim, Joseph J.** Effects of corticotropin on body water and electrolytes in patients with rheumatic disease 879
—. See CHALMERS, ECKHARDT, REYNOLDS, CIGARROA, DEANE, REIFENSTEIN, and SMITH *559*
Deiss, William P. See ALBRIGHT, LARSON, and DEISS *551*
De La Huerga, J., György, Paul, Waldstein, Sheldon, Katz, Ricardo, and **Popper, Hans.** The effects of antimicrobial agents upon choline degradation in the intestinal tract 1117
Dempsey, Eleanor F. See HENNEMAN, BARTTER, FORBES, and DEMPSEY *574*
Denny, Floyd W., Jr., Good, Robert A., and **Thomas, Lewis.** Experimental fibrinoid necrosis of the coronary arteries in streptococcus-infected rabbits *562*
—, and **Thomas, Lewis.** The demonstration of type specific streptococcal antibody by a hemagglutination technique employing tannic acid 1085
Dern, R. J. See WEINSTEIN, DERN, and TALMAGE *609*
Dickensheets, James. See HAVENS, DICKENSHEETS, BIERLY, and EBERHARD *573*
Dinning, James S. See OLSON and DINNING *592*
Dobriner, Konrad. Studies in steroid metabolism. XIX. The *a*-ketosteroid excretion pattern in normal males 940
—. Studies in steroid metabolism. XX. The reproducibility of the urinary steroid pattern in humans 950
Doig, Ronald K. See GRACE and DOIG *571*
Dole, Vincent P., Dahl, Lewis K., Schwartz, Irving L., Cotzias, George C., Thaysen, Jørn H., and **Harris, Cecilia.** Dietary treatment of hypertension. III. The effect of protein on appetite and weight 185
—. See SCHWARTZ, THAYSEN, and DOLE *602*
Dolger, Henry, Kupfer, Sherman, Bookman, John J., and **Carr, Julius.** Intravenous fructose in diabetes mellitus: Advantages over glucose in the treatment of diabetic emergencies *562*
Doolan, Paul D. See KYLE, JEGHERS, WALSH, DOOLAN, WISHINSKY, and PALLOTTA 364
Douglass, R. A., Jr. See ASSALI, KAPLAN, FOMON, and DOUGLASS 44
Dresdale, David T., Michtom, Robert J., and **Schultz, Martin.** Hemodynamic effects of lanatoside C in patients with no heart disease and in patients with heart disease but not in heart failure *563*
Dreskin, O. Herman. See ROSENTHAL, DRESKIN, and ROSENTHAL *599*
Duff, Robert S. Effect of surgical sympathectomy on the sensitivity to epinephrine of the blood vessels of muscular segments of the limbs 851
Dustan, Harriet P., Mason, Harold L., and **Corcoran, A. C.** Urinary formaldehydogenic corticoid as determined after enzymatic hydrolysis in normal subjects and in patients with adrenal and hypertensive disease 60

E

Earle, David P., Jr. See WERTHEIM, LYTTLE, LOEB, EARLE, SEEGAL, and SEEGAL 359
Easton, James G. See CULBERTSON, EASTON, and WELTON *561*
Ebaugh, Franklin G., Jr., Ross, J. F., and **Emerson, C. P.** The use of chromium 51 as an erythrocyte tagging agent for determining the survival of transfused and autotransfused erythrocytes *563*
—, **Emerson, Charles P.,** and **Ross, Joseph F.** The use of radioactive chromium 51 as an erythrocyte tagging agent for the determination of red cell survival in vivo 1260
—. See FINCH, ROSS, and EBAUGH *567*
Eberhard, Theodore P. See HAVENS, DICKENSHEETS, BIERLY, and EBERHARD *573*
Ebert, Richard V. See SCHULTZ, HAMMARSTEN, HELLER, and EBERT 107
—. See HAMMARSTEN, HELLER, and EBERT 340
—. See FRY and EBERT *570*
Eckert, Edward. See SCHOENBERGER, KARK, KROLL, and ECKERT *601*
Eckhardt, Richard D. See CHALMERS, ECKHARDT, REYNOLDS, CIGARROA, DEANE, REIFENSTEIN, and SMITH *559*
Eder, Howard A., and **Russ, Ella M.** Plasma protein-lipid relationships in acute hepatitis *564*
Ehrenhaft, Johann L. See BEDELL, CULBERTSON, EHRENHAFT, AMES, and THEILEN *554*
Eichna, Ludwig W. See FARBER and EICHNA 1140
—. See FARBER, BECKER, and EICHNA 1145
Eidinoff, Maxwell. See HELLMAN, ROSENFELD, FUKUSHIMA, EIDINOFF, BRUNSCHWIG, and GALLAGHER *573*
Eik-Nes, Kris. See TYLER, SANDBERG, and EIK-NES *608*
—. See BLISS, SANDBERG, NELSON, and EIK-NES 818

Einheber, Albert. See WILLIAMS, SHERWOOD, and EINHEBER *612*

Eisen, Herman N., Belman, Sidney, and **Carsten, Mary E.** Nature and localization of *in vivo* reactions between simple chemical allergens and tissue protein *564*

Eisenmenger, William J., and **Slater, Robert J.** Distribution and decay of I^{131} tagged albumin and gamma globulin in patients with cirrhosis *564*

Ek, Jan. See JOSEPHSON, EK, BUCHT, WERKÖ, KALLAS, and GRIEG 1184

Elkinton, J. R. See SINGER, ELKINTON, BARKER, and CLARK *604*

Elkinton, J. Russell. See SQUIRES, HUTH, and ELKINTON *605*

Ellison, Rose Ruth. See BURCHENAL, MURPHY, ELLISON, and KARNOFSKY *557*

Emerson, Charles P. See EBAUGH, ROSS, and EMERSON *563*

—. See EBAUGH, EMERSON, and ROSS 1260

Engel, Frank L. See WERK, HAMRICK, MYERS, and ENGEL *610*

—. See BURNS, ENGEL, VIAU, SCOTT, HOLLINGSWORTH, and WERK 781

Engstrom, William W., Markardt, Blanch, and **Liebman, Albert.** The influence of estrogens on the protein bound iodine of serum *565*

Epstein, Franklin H., Post, Robert S., and **McDowell, Marion.** The effect of an arteriovenous fistula on renal hemodynamics and electrolyte excretion 233

—, **Shadle, O. W., Ferguson, T. B.,** and **McDowell, M. E.** Cardiac output and intracardiac pressures in patients with arteriovenous fistulas 543

Estes, E. Harvey, Jr. See FITZHUGH, MCWHORTER, ESTES, WARREN, and MERRILL 1163

Etsten, Benjamin. See RELMAN, ETSTEN, and SCHWARTZ 972

Ettinger, Richard H., Giges, Burton, and **Waldstein, Sheldon S.** Glucose tolerance and glycogen deposition in experimental fatty liver *565*

—. See SMITH, ETTINGER, and SELIGSON *273*

Evans, Everett I. See JAMES, ABBOT, NORBERG, BIRKELAND, and EVANS *578*

F

Falk, Carolyn R., and **Appelbaum, Emanuel.** Type specific meningococcic agglutinins. III. Application of the test to sporadic cases and to clinically typical cases lacking bacteriological proof 39

Faloon, William W., and **Noll, Joan W.** Metabolic and hematologic studies in patients receiving chloramphenicol *565*

Farber, Saul J., and **Soberman, Robert J.** The relative amounts of body sodium and water in patients with heart, renal or hepatic disease *566*

—, and **Eichna, Ludwig W.** An intracardiac catheter with inflatable balloon for producing increased venous pressures in the venae cavae of man 1140

—, **Becker, William H.,** and **Eichna, Ludwig W.** Electrolyte and water excretions and renal hemodynamics during induced congestion of the superior and inferior vena cava of man 1145

Farr, Hollon W. See ROBERTS, RANDALL, and FARR *597*

Farr, Lee E. See GAMBLE, ROBERTSON, HANNIGAN, FOSTER, and FARR 483

Fazekas, Joseph F. See FINNERTY, WITKIN, and FAZEKAS *568*

Feldman, Felix. See LICHTMAN, WATSON, FELDMAN, GINSBERG, and ROBINSON 1229

Felts, John H., Jr. See AIKAWA, FELTS, and HARRELL 15

Fenninger, Leonard D. See WATERHOUSE, KEUTMANN, and FENNINGER 964

Ferber, M. Irene, Harvey, Réjane M., Samet, Philip, and **Cournand, André.** Myocardial and mechanical factors in mitral stenosis of rheumatic origin *566*

Ferguson, John H. See LEWIS and FERGUSON 915

Ferguson, T. B. See EPSTEIN, SHADLE, FERGUSON, and MCDOWELL 543

Field, John B., Zinn, Willard J., and **Griffith, George C.** Effect of heparin and a synthetic heparinoid on the physical state of serum lipids *566*

Field, William W. See CATHCART, FIELD, and RICHARDS 5

Fields, M. See BAUER, BLAHD, and FIELDS *553*

Figueroa, William G. See ADAMS and FIGUEROA *550*

Finch, Clement A., and **Coleman, Daniel H.** Patterns of erythropoiesis in hemolytic anemia *567*

Finch, Stuart C., Ross, Joseph F., and **Ebaugh, Franklin G., Jr.** Immunologic mechanisms of leukocyte abnormalities *567*

Finkenstaedt, John T., O'Meara, Maurice P., and **Merrill, John P.** Observations on the volume of distribution of inulin in anuric subjects 209

—, —, **Weller, John M.,** and **Merrill, John P.** Renal function after recovery from acute renal failure *567*

Finnerty, Frank A., Jr., Witkin, Lloyd, and **Fazekas, Joseph F.** Cerebral hemodynamics in acute induced postural hypotension *568*

—. See FREIS, ROSE, FINNERTY, and PARTENOPE *569*

Fischel, Edward E. Serum complement as an indication of the presence and degree of inflammatory reaction in various diseases *568*

Fishman, Robert A. The failure of intracranial pressure-volume change to influence renal function 847

Fishman, William H., Dart, R. M., Bonner, C. D., Leadbetter, W. F., Lerner, F., and **Homburger, F.** A new method for estimating serum acid phosphatase of prostatic origin applied to the clinical investigation of cancer of the prostate 1034

—. See HOMBURGER and FISHMAN *575*

Fitzhugh, Francis W., Jr., McWhorter, Robert L., Jr., Estes, E. Harvey, Jr., Warren, James V., and **Merrill, Arthur J.** The effect of application of tourniquets to the legs on cardiac output and renal function in normal human subjects 1163

Fitzpatrick, H. F. See BRADLEY, SMYTHE, FITZPATRICK, and BLAKEMORE 526

Fitzgerald, J. C. See PARKER, FITZGERALD, PERRY, and WOODWARD *593*

Fleisher, Gerard A., and **Butt, Hugh R.** Peptidases in human serum: A comparison of activities between normal persons and patients with liver diseases and other pathologic conditions 674

Flink, Edmund B., Stutzman, Francis L., Anderson, Arthur R., and **Konig, Theodore J.** Magnesium deficiency in delirium tremens *568*

Folette, James H. See VALENTINE, FOLLETTE, and LAWRENCE 251

Fomon, S. J. See ASSALI, KAPLAN, FOMON, and DOUGLASS 44

Forbes, Anne P. See HENNEMAN, BARTTER, FORBES, and DEMPSEY *574*
Ford, Ralph V., Moyer, John H., and **Spurr, Charles L.** Hexamethonium in the chronic treatment of hypertension—its effect on renal hemodynamics and on the excretion of water and electrolytes 1133
Forsham, P. H. See LIDDLE, RINFRET, ISLAND, and FORSHAM *584*
Forsham, Peter H. See LIDDLE, BENNETT, and FORSHAM 1197
Foster, Charles G. See GAMBLE, ROBERTSON, HANNIGAN, FOSTER, and FARR 483
Frank, Heddy, and **Gray, Seymour J.** The determination of plasma volume in man with radioactive chromic chloride 991
—. See GRAY and FRANK *571*
—. See GRAY and FRANK 1000
Frawley, Thomas F. Some effects of alteration in body electrolytes on saliva electrolyte composition *569*
Freedberg, A. Stone. See HAMOLSKY, FREEDBERG, KURLAND, and WOLSKY 453
Freeman, M. See REYNOLDS, PATON, FREEMAN, HOWARD, and SHERLOCK 793
Freeman, N. E. See BIERMAN, BYRON, KELLY, GILFILLAN, WHITE, FREEMAN, and PETRAKIS 637
Freinkel, Norbert, Schreiner, George E., and **Athens, John W.** Simultaneous distribution of T-1824 and I^{131}-labelled human serum albumin in man 138
—, and **Ingbar, Sidney H.** Concentration gradients for inorganic I^{131} and chloride in mixed human saliva 1077
Freis, Edward D., Rose, John C., Finnerty, Frank A., Jr., and **Partenope, Edward A.** A comparative study of the hemodynamic alterations produced by hexamethonium and l-hydrazinophthalazine (apresoline) in hypertensive patients *569*
—, —, **Partenope, Edward A., Higgins, Thomas F., Kelley, Robert T., Schnaper, Harold W.,** and **Johnson, Robert L.** The hemodynamic effects of hypotensive drugs in man. III. Hexamethonium 1285
French, Frank S. See ST. GEORGE, NAEGELE, FRENCH, ROSENMAN, and FRIEDMAN 1222
Friedland, Carl K. See HAFKENSCHIEL and FRIEDLAND 655
Friedman, Irwin H. See JUDSON, HOLLANDER, FRIEDMAN, HATCHER, and HALPERIN *579*
Friedman, Meyer, and **St. George, Shirley.** The cardiac and hepatic intracellular fate of digitoxin *569*
—. See ROSENMAN, FRIEDMAN, and BYERS 121
—. See ST. GEORGE, NAEGELE, FRENCH, ROSENMAN, and FRIEDMAN 1222
Fry, Donald L., and **Ebert, Richard V.** The mechanics of pulmonary ventilation in emphysema *570*
Fukushima, David. See HELLMAN, ROSENFELD, FUKUSHIMA, EIDINOFF, BRUNSCHWIG, and GALLAGHER *573*

G

Gabiga, Alfreda M. See IZZO, HOFFMASTER, and GABIGA *577*
Gabuzda, George J., Jr., Gocke, Thomas M., and **Love, Ben D., Jr.** Metabolic effects of antibiotics administered to undernourished men *570*
—, **Phillips, Gerald B.,** and **Davidson, Charles S.** Nutritive adequacy and clinical tolerance of modified human globin 899
—. See SCHWARTZ, SEEGMILLER, PHILLIPS, GABUZDA, and DAVIDSON *602*
Gallagher, Thomas. See HELLMAN, ROSENFELD, FUKUSHIMA, EIDINOFF, BRUNSCHWIG, and GALLAGHER *573*
Gamble, James L., Jr., Robertson, James S., Hannigan, Charles A., Foster, Charles G., and **Farr, Lee E.** Chloride, bromide, sodium, and sucrose spaces in man 483
Ganzell, Mary Ann. See JESSAR, GANZELL, and RAGAN 480
Garcia-Reyes, Jose A. See JENKINS, GARCIA-REYES, and MCCRACKEN *579*
Gardner, Frank H. See ROBIN and GARDNER *598*
Gautier, E. See MCARTHUR, SMART, HARTING, GAUTIER, GODLEY, SWALLOW, SIMEONE, and HUME *587*
Giges, Burton. See ETTINGER, GIGES, and WALDSTEIN *565*
—. See SBOROV, GIGES, and MANN *601*
Gilder, H. See SLEISENGER, ALMY, GILDER, and PERLE 466
Gilfillan, R. S. See BIERMAN, BYRON, KELLY, GILFILLAN, WHITE, FREEMAN, and PETRAKIS 637
Gilmore, J. P. See SMYTHE, GILMORE, and HANDFORD *605*
Ginsberg, Victor. See LICHTMAN, WATSON, FELDMAN, GINSBERG, and ROBINSON 1229
Glyn, John. See ZIFF, WILSON, SCIALABBA, SCULL, and GLYN *614*
Gocke, Thomas M. See GABUZDA, GOCKE, and LOVE *570*
Godley, A. See MCARTHUR, SMART, HARTING, GAUTIER, GODLEY, SWALLOW, SIMEONE, and HUME *587*
Goger, Pauline R. See JOHNSON and GOGER *579*
Gold, Jay J. See JAILER, LOUCHART, GOLD, and KNOWLTON 449
—. See JAILER, GOLD, and LIEBERMAN *578*
Goldstein, Robert. See ALEXANDER and GOLDSTEIN *551*
Goluboff, Bernard. See SHENKIN, NOVACK, GOLUBOFF, SOFFE, and BORTIN 459
—. See NOVACK, SHENKIN, BORTIN, GOLUBOFF, and SOFFE 696
Good, Robert A. See DENNY, GOOD, and THOMAS *562*
Goodyer, Allan V. N., and **Seldin, Donald W.** The effects of quiet standing on solute diuresis 242
—, —. The excretion of solutes by patients with edema *570*
Gordillo, G. See METCOFF and GORDILLO *590*
Gordon, Ida. See CURRENS, KHAMBATTA, CRANLEY, and GORDON *561*
Gordon, M. See LIPSKY, ALPER, RUBINI, GORDON, and VAN ECK *584*
Grace, William J., and **Doig, Ronald K.** The relationship between gastric acidity and the absorption of iron from the upper gastrointestinal tract *571*
Gray, Seymour J., and **Frank, Heddy.** The selective tagging of red blood cells and plasma proteins with radioactive sodium chromate and chromic chloride: Its use in the simultaneous determination of red cell mass and plasma volume in man *571*
—, —. The simultaneous determination of red cell mass and plasma volume in man with radioactive sodium chromate and chromic chloride 1000
—. See FRANK and GRAY 991
Green, R. See MARTIN, MCKINNEY, and GREEN *586*
—. See MARTIN, MCKINNEY, GREEN, and BECKER 1171

Green, Richard W. The effects of platelet and thrombin concentration on fibrin strand width *571*
Greene, David G. Spirography in the evaluation of pulmonary function prior to surgery *571*
Greenman, Lawrence, Weigand, F. A., and Danowski, T. S. Prevention of cardiac abnormalities in acute rheumatic carditis *572*
Grieg, Arne. See Josephson, Ek, Bucht, Werkö, Kallas, and Grieg 1184
Griffith, George C. See Field, Zinn, and Griffith *566*
Grob, David, and McKusick, Victor A. Cardiovascular and renal effects of hexamethonium and 1-hydrazinophthalazine in hypertensive patients *572*
Grollman, Arthur, and Shapiro, Alvin P. The volume of the extracellular fluid in experimental and human hypertension 312
—. See Walser, Seldin, and Grollman 299
Grossman, Jacob, Weston, Raymond E., and Leiter, Louis. A method for determining cardiac output by the direct Fick principle without gas analysis 161
—. See Bernstein, Weston, Ross, Grossman, Hanenson, and Leiter 422
—. See Weston, Hanenson, Grossman, Berdasco, and Wolfman *611*
Gubler, C. J., Lahey, M. E., Cartwright, G. E., and Wintrobe, M. M. Studies on copper metabolism 405
—. See Lahey, Gubler, Cartwright, and Wintrobe 322
—. See Lahey, Gubler, Cartwright, and Wintrobe 329
Guest, George M. See Knowles and Guest *580*
Gutman, Alexander B. See Benedict, Yü, Bien, Gutman, and Stetten 775
—. See Bien, Yü, Benedict, Gutman, and Stetten 778
—. See Yü, Sirota, and Gutman 1121
György, Paul. See De La Huerga, György, Waldstein, Katz, and Popper 1117

H

Hafkenschiel, Joseph H., and Friedland, Carl K. The effects of 1-hydrazinophthalazine on cerebral blood flow, vascular resistance, oxygen uptake, and jugular oxygen tension in hypertensive subjects 655
Hagans, James A. See Brust, Hagans, and Reiser *556*
Hall, Wendell H., and Manion, Robert E. Comparison of the Coombs test with other methods for brucella agglutinins in human serum 96
Halperin, Meyer H. See Judson, Hollander, Friedman, Hatcher, and Halperin *579*
Hamburger, Morton, and Biehl, J. Park. Some effects of injecting sterile solutions of streptokinase-streptodornase into the subarachnoid space of normal rhesus monkeys 391
—, and **Lemon, Henry M.** The failure of antistreptolysin to protect against scarlet fever or streptococcal pharyngitis *572*
Hammarsten, James F., Heller, Ben I., and Ebert, Richard V. The effects of dextran in normovolemic and oligemic subjects 340
—. See Schultz, Hammarsten, Heller, and Ebert 107
—. See Heller, Hammarsten, and Stutzman 858
Hamolsky, Milton W., Freedberg, A. Stone, Kurland, George S., and Wolsky, Leonard. The exchangeable thyroid hormonal pool. I. Its magnitude and rate of turnover in various thyroid states in man 453
Hamrick, Ladd W., Jr., and Myers, J. D. The effect of acute hemorrhage on the hepatic blood flow and splanchnic oxygen consumption of the dog *573*
—. See Werk, Hamrick, Myers, and Engel *610*
Handford, S. W. See Smythe, Gilmore, and Handford *605*
Hanenson, Irwin B. See Bernstein, Weston, Ross, Grossman, Hanenson, and Leiter 422
—. See Weston, Hanenson, Grossman, Berdasco, and Wolfman *611*
Hannigan, Charles A. See Gamble, Robertson, Hannigan, Foster, and Farr 483
Harrell, George T., Jr. See Aikawa, Felts, and Harrell 15
Harris, Cecilia. See Dole, Dahl, Schwartz, Cotzias, Thaysen, and Harris 185
Harris, J. F., Lloyd, C. W., and Lobotsky, J. Some studies of posterior pituitary and adrenal cortical interrelationships in patients with and without cirrhosis of the liver 885
Harting, D. See McArthur, Smart, Harting, Gautier, Godley, Swallow, Simeone, and Hume *587*
Harvey, Réjane M. See Ferrer, Harvey, Samet, and Cournand *566*
Harvey, W. Proctor. See Rose, Hufnagel, Harvey, and Partenope *598*
Hatcher, J. D. See Judson, Hollander, Friedman, Hatcher, and Halperin *579*
Havens, W. Paul, Jr., Dickensheets, James, Bierly, James N., and Eberhard, Theodore P. The half-life of normal human gamma globulin in patients with hepatic cirrhosis *573*
Hein, David E. See Billing, Conlon, Hein, and Schiff 214
Hellbaum, Arthur A. See Shetlar, Payne, Bullock, Patrick, Hellbaum, and Ishmael 1208
Heller, B. I., Hammarsten, J. F., and Stutzman, F. L. Concerning the effects of magnesium sulfate on renal function, electrolyte excretion, and clearance of magnesium 858
Heller, Ben I. See Schultz, Hammarsten, Heller, and Ebert 107
—. See Hammarsten, Heller, and Ebert 340
Hellman, Leon, Rosenfeld, Robert, Fukushima, David, Eidinoff, Maxwell, Brunschwig, Alexander, and Gallagher, Thomas. The major pathways of steroid metabolism in man *573*
Henneman, Philip H., Bartter, Frederic C., Forbes, Anne P., and Dempsey, Eleanor F. The effect of intravenously administered human serum albumin on calcium metabolism in osteoporosis *574*
Heyman, Albert, and Patterson, John L., Jr. The cerebral circulation and metabolism in hypertensive and arteriosclerotic cerebral vascular disease, with observations on the effects of oxygen inhalation *574*
Hickam, J. B. See Burnum, Hickam, and McIntosh *558*
—. See McIntosh, Burnum, Hickam, and Warren *588*
Higgins, Thomas F. See Freis, Rose, Partenope, Higgins, Kelley, Schnaper, and Johnson 1285

Hills, A. Gorman, Chalmers, Theodore N., and **Webster, George D.** Redistribution of water and electrolytes in acute adrenal cortical insufficiency in man *574*

—, —, **Webster, G. D., Jr.,** and **Rosenthal, Otto.** Adrenal cortical regulation of the distribution of water and electrolytes in the human body 1236

Hinkle, Lawrence E., Jr. See SCHOTTSTAEDT and HINKLE *602*

Hirsch, Marvin M. See LEPPER, JACKSON, and HIRSCH *582*

Hodes, Horace L. See KATCHER, LEVITT, SWEET, and HODES 1013

Hodges, Robert E., Paul, William D., Routh, Joseph I., Daum, Kate, and **Bradbury, James T.** Metabolic aspects of rheumatoid arthritis as influenced by nitrogen mustard *575*

Hoffmaster, Joanne. See IZZO, HOFFMASTER, and GABIGA *577*

Hollander, William. See JUDSON, HOLLANDER, FRIEDMAN, HATCHER, and HALPERIN *579*

Holliday, Malcolm A. Factors controlling urinary cation excretion in chloride deficiency *575*

Hollingsworth, Dorothy R. See BURNS, ENGEL, VIAU, SCOTT, HOLLINGSWORTH, and WERK 781

Homburger, F., and **Fishman, W. H.** Clinical experience with a new method for estimating serum acid phosphatase of prostatic origin *575*

—. See FISHMAN, DART, BONNER, LEADBETTER, LERNER, and HOMBURGER 1034

Horstmann, Dorothy M., and **McCollum, Robert W.** Viremia in human poliomyelitis *576*

Horwitz, Orville. See WILLIAMS, MONTGOMERY, and HORWITZ 1097

Howard, F. See REYNOLDS, PATON, FREEMAN, HOWARD, and SHERLOCK 793

Howes, Edward L. See MACLENNAN, MANDL, and HOWES 1317

—. See MANDL, MACLENNAN, and HOWES 1323

Hufnagel, Charles A. See ROSE, HUFNAGEL, HARVEY, and PARTENOPE *598*

Hughes, Felix. See RICE and HUGHES *597*

Hume, D. See MCARTHUR, SMART, HARTING, GAUTIER, GODLEY, SWALLOW, SIMEONE, and HUME *587*

Hurley, Thomas H., and **Weisman, Russell, Jr.** Studies in survival and separation of transfused red cells by differential agglutination compared to a method of differential hemolysis *576*

Huth, Edward J. See SQUIRES, HUTH, and ELKINTON *605*

I

Ingbar, Sidney H. A new method for the study of thyroidal function in man, using radioactive iodine *577*

—. See FREINKEL and INGBAR 1077

Ingenito, Estelle F. See RUTSTEIN, INGENITO, and BOURNEUF *600*

Inkley, Scott R., Krieger, Harvey, and **Brooks, Laura.** A study of methods for the prediction of plasma volume in fifty normal subjects *577*

Isenberg, S. Allen. See STEINBROCKER, ISENBERG, SILVER, NEUSTADT, KUHN, and SCHITTONE 1045

Ishmael, William K. See SHETLAR, PAYNE, BULLOCK, PATRICK, HELLBAUM, and ISHMAEL 1208

Island, D. See LIDDLE, RINFRET, ISLAND, and FORSHAM *584*

Izzo, Joseph L., Hoffmaster, Joanne, and **Gabiga, Alfreda M.** Metabolic studies in diabetes mellitus. Blood sugar level and urinary excretion of sugar and nitrogen in relation to exogenous insulin in patients with unstable and stable diabetes *577*

J

Jackson, George Gee. See LEPPER, JACKSON, and HIRSCH *582*

Jacox, Ralph F. Quantitative fractionation of component proteins of human serum with cationic detergents 661

Jailer, Joseph W., Louchart, Jean, Gold, Jay J., and **Knowlton, A. I.** The effect of cortisone on the urinary excretion of 17-ketosteroids in patients with Cushing's syndrome 449

—, **Gold, Jay J.,** and **Lieberman, Seymour.** The effect of cortisone upon the urinary 17-ketosteroid excretion in various adrenal states *578*

James, G. Watson, III, Abbot, Lynn D., Jr., Norberg, Bo, Birkeland, Steffen, and **Evans, Everett I.** Metabolic fate of N^{15}-labelled erythrocytes transfused into normal man *578*

James, J. A. See METCOFF, JAMES, and GORDILLO *590*

Jansen, M. T. A reflection spectrophotometric study of ultraviolet erythema and pigmentation 1053

Jawetz, Ernest. The influence of cortisone on the efficacy of antibiotic therapy of experimental infections *578*

Jefferies, William McK. See LEVY, KELLY, and JEFFERIES *583*

Jeghers, Harold. See KYLE, JEGHERS, WALSH, DOOLAN, WISHINSKY, and PALLOTTA 364

Jenkins, Dalton, Garcia-Reyes, Jose A., and **McCracken, Brian H.** Intravenous adrenal cortical steroids *579*

—. See LAIDLAW and JENKINS *581*

Jenson, Robert L. See SCHWARTZ, JENSON, and RELMAN *602*

Jessar, Ralph A., Ganzell, Mary Ann, and **Ragan, Charles.** The action of hydrocortisone in synovial inflammation 480

Johnson, Alan J., and **Goger, Pauline R.** The intravenous injection into patients of crystalline pancreatic desoxyribonuclease. Its excretion in the urine and its distribution in certain body fluids *579*

Johnson, Ben B. See LUETSCHER and JOHNSON *585*

Johnson, Robert L. See FREIS, ROSE, PARTENOPE, HIGGINS, KELLY, SCHNAPER, and JOHNSON 1285

Joos, Howard A. See YU, LOVEJOY, JOOS, NYE, and MCCANN 130

—. See LOVEJOY, YU, NYE, JOOS, and SIMPSON *585*

Josephson, Bertil, Ek, Jan, Bucht, Härje, Werkö, Lars, Kallas, Julia, and **Grieg, Arne.** The competition between p-amino-hippuric acid (PAH) and diodrast for renal excretion and extraction in man 1184

Judson, Walter E., Hollander, William, Friedman, Irwin H., Hatcher, J. D., and **Halperin, Meyer H.** Studies on congestive failure: The effects of exercise, salt loading and of intravenous apresoline on cardiorenal hemodynamics and the excretion of sodium and water *579*

—. See WILKINS, TINSLEY, CULBERTSON, BURROWS, JUDSON, and BURNETT 1101

K

Kallas, Julia. See Josephson, Ek, Bucht, Werkö, Kallas, and Grieg 1184

Kantor, Thomas. See Ziff, Kantor, Bien, and Smith 1253

Kaplan, S. A. See Assali, Kaplan, Fomon, and Douglass 44

—. See Assali, Kaplan, Oighenstein, and Suyemoto 922

Karnofsky, David A. See Burchenal, Murphy, Ellison, and Karnofsky 557

Kark, Robert M. See Schoenberger, Kark, Kroll, and Eckert 601

Karl, Michael M. See Shank, Mendeloff, and Karl 603

Katcher, Avrum L., Levitt, Marvin F., Sweet, Avron Y., and **Hodes, Horace L.** Alterations of fluid and electrolyte distribution and renal function in diarrhea of infancy 1013

Katz, Ricardo. See De La Huerga, György, Waldstein, Katz, and Popper 1117

Keating, F. Raymond, Jr., and **Albert, A.** Distribution and metabolism of radioactive l-triiodothyronine compared with radioactive l-thyroxine in the rat 580

Kelly, Keith H. See Bierman, Cordes, Kelly, Littman, Steinfeld, and Yamauchi 555

—. See Bierman, Byron, Kelly, Gilfillan, White, Freeman, and Petrakis 637

Kelly, Luther W., Jr. See Levy, Kelly, and Jefferies 583

Kelley, Robert T. See Freis, Rose, Partenope, Higgins, Kelley, Schnaper, and Johnson 1285

Kennamer, S. Rexford. See Prinzmetal, Kennamer, Shaw, Maxwell, and Bernstein 595

Kennedy, Thomas J., Jr., and **Kleh, Jack.** The relationship between the clearance and the plasma concentration of inulin in normal man 90

—. See Orloff, Kennedy, and Berliner 538

Kety, Seymour S. See Sokoloff, Wechsler, Mangold, Balis, and Kety 202

Keutmann, E. Henry. See Waterhouse, Keutmann, and Fenninger 964

Khambatta, R. B. See Currens, Khambatta, Cranley, and Gordon 561

Kibrick, Sidney. See Miller, Kibrick, and Massell 691

Kilbourne, Edwin D., and **Tateno, Isao.** Influence of corticosteroid hormones on infection in vitro 580

Kleeman, Charles R., Bass, David E., and **Quinn, Murray.** The effect of an impermeable vapor barrier on electrolyte and nitrogen concentrations in sweat 736

Kleh, Jack. See Kennedy and Kleh 90

Knott, John R. See Lewis, Sinton, and Knott 583

Knowles, Harvey C., Jr., and **Guest, George M.** Changes of tissue electrolytes in diabetic acidosis 580

Knowlton, Abbie I., Loeb, Emily N., Stoerk, Herbert C., and **Mohler, William C.** The combined effect of cortisone acetate and desoxycorticosterone acetate in adrenalectomized rats on a low sodium diet 1175

—. See Jailer, Louchart, Gold, and Knowlton 449

Kohen, Roland J. See Stetson, Rammelkamp, Perry, Krause, and Kohen 606

Konig, Theodore J. See Flink, Stutzman, Anderson, and Konig 568

Korpman, R. See Lorant, Lorant, Angrist, and Korpman 1005

Kossmann, Charles E., Briller, Stanley A., and **Marchand, Nathan.** Electrical energetics of the myocardium in man 581

Kowalski, Henry J., and **Abelmann, Walter H.** The cardiac output at rest in Laennec's cirrhosis 1025

—. See Abelmann and Kowalski 550

Krause, Richard M. See Stetson, Rammelkamp, Perry, Krause, and Kohen 606

Krieger, Harvey. See Inkley, Krieger, and Brooks 577

Kroll, George. See Schoenberger, Kark, Kroll, and Eckert 601

Kuhn, Paul. See Steinbrocker, Isenberg, Silver, Neustadt, Kuhn, and Schittone 1045

Kukral, John. See Armstrong, McLeod, Wolter, and Kukral 552

Kuna, Andrew, and **Chambers, Robert.** The effect of saccharides on leukocyte movement 436

Kupfer, Sherman. See Dolger, Kupfer, Bookman, and Carr 562

Kurland, George S. See Hamolsky, Freedberg, Kurland, and Wolsky 453

Kurnick, Nathaniel B., Schwartz, Lawrence I., Pariser, Sanford, and **Lee, Stanley L.** A specific inhibitor for human desoxyribonuclease and an inhibitor of the lupus erythematosus cell phenomenon from leucocytes 193

Kurnick, N. B. See Carrerea, Reid, and Kurnick 559

Kurtz, Martin. See White, Kurtz, and Rubin 611

Kyle, Laurence H., Jeghers, Harold, Walsh, William P., Doolan, Paul D., Wishinsky, Henry, and **Pallotta, Arthur.** The application of hemodialysis to the treatment of barbiturate poisoning 364

L

Lahey, M. E., Gubler, C. J., Cartwright, G. E., and **Wintrobe, M. M.** Studies on copper metabolism. VI. Blood copper in normal human subjects 322

—, —, —, —. Studies on copper metabolism. VII. Blood copper in pregnancy and various pathologic states 329

—. See Gubler, Lahey, Cartwright, and Wintrobe 405

Laidlaw, John C., and **Jenkins, Dalton.** Stress and eosinophils 581

Lanchantin, Gerald F., and **Ware, Arnold G.** Identification of a thromboplastin inhibitor in serum and in plasma 381

Lange, Kurt, Slobody, Lawrence B., and **Strang, Ruth H.** Serum complement levels as a guide for diagnosis and therapy of the nephrotic syndrome 581

Larson, Daniel L., and **Tomlinson, Lois J.** Quantitative antibody studies in man. III. Antibody response in leukemia and other malignant lymphomata 317

—, and **Ranney, Helen M.** Filter paper electrophoresis of human hemoglobin 1070

—. See Ranney, Larson, and McCormack 1277

Larson, Frank C. See Albright, Larson, and Deiss 551

Laszlo, Daniel. See Lewin, Spencer, and Laszlo 584

Lathem, Willoughby, Roof, Betty S., and **Nickel, James F.** The effect of the upright position on protein excretion and renal function in renal disease 582

Lawrence, John S. See VALENTINE, FOLLETTE, and LAWRENCE 251
Leach, Robert B. See MADDOCK, TOKUYAMA, and LEACH *586*
Leadbetter, Wyland F. See D'AGOSTINO, LEADBETTER, and SCHWARTZ 444
—. See FISHMAN, DART, BONNER, LEADBETTER, LERNER, and HOMBURGER 1034
Leaf, Alexander, Bartter, Frederic C., Santos, Roberto F., and **Wrong, Oliver.** The role of water intake in producing or abolishing diuresis of sodium resulting from pitressin administration in man *582*
—, —, —, —. Evidence in man that urinary electrolyte loss induced by pitressin is a function of water retention 868
—. See MARKS and LEAF 813
Lee, Helen C. See BERLIN, TOLBERT, and LEE 1
Lee, Stanley L. See KURNICK, SCHWARTZ, PARISER, and LEE 193
Leifer, Edgar. See NICKEL, LOWRANCE, LEIFER, and BRADLEY 68
Leiter, Louis. See GROSSMAN, WESTON, and LEITER 161
—. See BERNSTEIN, WESTON, ROSS, GROSSMAN, HANENSON, and LEITER 422
—. See WHITE, RUBIN, and LEITER 931
Lemon, Henry M. See HAMBURGER and LEMON *572*
Lepper, Mark H., Jackson, George Gee, and **Hirsch, Marvin M.** Epidemiology of penicillin and aureomycin resistant staphylococci in a hospital community *582*
Lerner, F. See FISHMAN, DART, BONNER, LEADBETTER, LERNER, and HOMBURGER 1034
Levenson, Stanley M., Adams, Margaret A., Rosen, Hyman, and **Taylor, F. H. Laskey.** Studies in phosphorus metabolism in man. III. The distribution, exchange, and excretion of phosphorus in man using radioactive phosphorus (P^{32}) as a tracer 497
Levine, Bennett. See WEISBERGER and LEVINE *610*
Levitt, Marvin F., and **Turner, Louis B.** Role of bone, connective tissue and muscle electrolytes in the maintenance of extracellular fluid composition. *583*
—. See KATCHER, LEVITT, SWEET, and HODES 1013
Levy, Richard P., Kelly, Luther W., Jr., and **Jefferies, William McK.** The study of thyroid function by means of a single injection of thyrotropin *583*
Lewin, Isaac, Spencer, Herta, and **Laszlo, Daniel.** Metabolism of bone disease studies by the calcium tolerance test *584*
Lewis, Bernard I., Sinton, David W., and **Knott, John R.** Central nervous system involvement in the disorders of collagen *583*
Lewis, Jessica H., and **Ferguson, John H.** Partial purification of human prothrombin and proconvertin: Their characteristics and interaction 915
Lichtman, Herbert C., Watson, R. Janet, Feldman, Felix, Ginsberg, Victor, and **Robinson, Jean.** Studies on thalassemia. Part I. An extracorpuscular defect in thalassemia major. Part II. The effects of splenectomy in thalassemia major with an associated acquired hemolytic anemia 1229
Liddle, Grant W., Rinfret, A. P., Island, D., and **Forsham, P. H.** Factors responsible for the variable effectiveness of ACTH in man *584*
—, **Bennett, Leslie L.,** and **Forsham, Peter H.** The prevention of ACTH-induced sodium retention by the use of potassium salts: A quantitative study 1197
Lieberman, Seymour. See JAILER, GOLD, and LIEBERMAN *578*
Liebman, Albert. See ENGSTROM, MARKARDT, and LIEBMAN *565*
Linenthal, Arthur J. See ZOLL, NORMAN, and LINENTHAL *614*
Lipsky, S. R., Alper, B. J., Rubini, M., Gordon, M., and **Van Eck.** The effects of sodium acetate administration upon ketone body production in normal and diabetic subjects *584*
Littman, Aline M. See BIERMAN, CORDES, KELLY, LITTMAN, STEINFELD, and YAMAUCHI *555*
Livesay, W. R. See CHAPMAN and LIVESAY *560*
Lloyd, C. W. See HARRIS, LLOYD, and LOBOTSKY 885
Lobotsky, J. See HARRIS, LLOYD, and LOBOTSKY 885
Loeb, Emily N. See WERTHEIM, LYTTLE, LOEB, EARLE, SEEGAL, and SEEGAL 359
—. See KNOWLTON, LOEB, STOERK, and MOHLER 1175
Logsdon, George R. See TARAIL and LOGSDON *607*
London, Irving M., and **Schwarz, Haiyim.** Erythrocyte metabolism. The metabolic behavior of the cholesterol of human erythrocytes 1248
Lorant, A., Lorant, G. J., Angrist, A., and **Korpman, R.** Storage of blood below 0° C. in liquid state 1005
Lorant, G. J. See LORANT, LORANT, ANGRIST, and KORPMAN 1005
Louchart, Jean. See JAILER, LOUCHART, GOLD, and KNOWLTON 449
Love, Ben D., Jr. See GABUZDA, GOCKE, and LOVE *570*
Lovejoy, Frank W., Jr., Yu, Paul N. G., Nye, Robert E., Jr., Joos, Howard A., and **Simpson, John H.** Physiologic and hemodynamic studies of patients with CO_2 narcosis *585*
—. See YU, LOVEJOY, JOOS, NYE, and MCCANN 130
Lowrance, Preston B. See NICKEL, LOWRANCE, LEIFER, and BRADLEY 68
Lowry, Paul T. The isolation of crystalline d-urobilinogen and its probable significance *585*
Luetscher, John A., Jr., and **Johnson, Ben B.** The sodium-retaining corticoid of human urine: Its nature and some physiological factors governing its secretion *585*
Luft, Rolf, and **Von Euler, Ulf S.** Two cases of postural hypotension showing a deficiency in release of non-epinephrine and epinephrine 1065
Lukas, Daniel S. See CARDON and LUKAS *559*
Lyttle, John D. See WERTHEIM, LYTTLE, LOEB, EARLE, SEEGAL, and SEEGAL 359

M

Mack, Marian H., Wolf, Stewart, and **Stern, Kurt G.** Preliminary electrophoretic analysis of human gastric juice 862
MacLennan, John D., Mandl, Ines, and **Howes, Edward L.** Bacterial digestion of collagen 1317
—. See MANDL, MACLENNAN, and HOWES 1323
Maddock, William O., Tokuyama, Ichiro, and **Leach, Robert B.** Antihormone formation in patients receiving hog pituitary FSH; possible therapeutic application *586*
Madison, Leonard. See SENSENBACH, MADISON, and OCHS 226
—. See SENSENBACH, MADISON, and OCHS 372

Mandl, Ines, MacLennan, John D., and Howes, Edward L. Isolation and characterization of proteinase and collagenase from CL. Histolyticum 1323
—. See MacLennan, Mandl, and Howes 1317
Mangold, Renward. See Sokoloff, Wechsler, Mangold, Balls, and Kety 202
Manion, Robert E. See Hall and Manion 96
Mann, Joseph. See Sborov, Giges, and Mann *601*
Marchand, Nathan. See Kossmann, Briller, and Marchand *581*
Markardt, Blanch. See Engstrom, Markardt, and Liebman *565*
Marks, Leon J., and **Leaf, Alexander.** The relationship of the renal excretion of adrenal corticoids to variations in renal hemodynamics 813
Marshall, Helen. See Martin, Cline, and Marshall 617
Martin, C. J., Cline, Frank, Jr., and **Marshall, Helen.** Lobar alveolar gas concentrations: effect of body position 617
Martin, Samuel P., McKinney, Gordon R., and **Green, R.** Effect of glucose, fructose, and insulin on the leukocytes of diabetics *586*
—, —, —, and **Becker, Caroline.** The influence of glucose, fructose, and insulin on the metabolism of leukocytes of healthy and diabetic subjects 1171
Mason, Harold L. See Dustan, Mason, and Corcoran 60
Masouredis, S. P. See Petrakis, Masouredis, and Miller 952
Massell, Benedict F. See Miller, Kibrick, and Massell 691
Massey, B. W. See Rubin, Massey, Stonecypher, and Dobson *600*
Maxwell, Morton. See Prinzmetal, Kennamer, Shaw, Maxwell, and Bernstein *595*
McArthur, J. W., Smart, G. A., Harting, D., Gautier, E., Godley, A., Swallow, K. A., Simeone, F. A., and **Hume, D.** The role of the alarm reaction in the pathologic physiology of experimental diabetic acidosis *587*
McCann, William S. See Yu, Lovejoy, Joos, Nye, and McCann 130
McCleod, Katherine. See Armstrong, McLeod, Wolter, and Kukral *552*
McCollum, Robert W. See Horstmann, and McCollum *576*
McCormack, George H., Jr. See Turner, McCormack, and Richards 801
—. See Ranney, Larson, and McCormack 1277
McCracken, Brian H. See Jenkins, Garcia-Reyes, and McCracken *579*
McCune, Robert M. The behavior of populations of tubercle bacilli in vivo *587*
McDermott, William, and **Adams, Raymond D.** Eckfistula.—A cause of episodic stupor in humans *587*
McDonald, Lawson, and **Rabinowitz, Murray.** Cardiac output and pulmonary arteriolar resistance in mitral stenosis and mitral incompetence *588*
—. See Rabinowitz and McDonald *595*
McDowell, Marion. See Epstein, Post, and McDowell 233
McDowell, M. E. See Epstein, Shadle, Ferguson, and McDowell 543
McIntosh, B. J. See Brodsky, Rehm, and McIntosh *556*
McIntosh, H. D., Burnum, J. F., Hickam, J. B., and **Warren, J. V.** Observations on the circulatory phenomena produced by the valsalva maneuver *588*
—. See Burnum, Hickam, and McIntosh *558*
McKenna, Roger. See Crosley, Rowe, McKenna, and Crumpton *561*
McKinney, Gordon R. See Martin, McKinney, and Green *586*
—. See Martin, McKinney, Green, and Becker 1171
McKusick, Victor A. See Grob and McKusick *572*
McWhorter, Robert L., Jr. See Fitzhugh, McWhorter, Estes, Warren, and Merrill 1163
Mead, Jere, and **Whittenberger, James L.** Clinical measurement of resistive and elastic properties of the lungs *588*
Meilman, Edward. Clinical studies on veratrum alkaloids. III. The effect of protoveratrine on renal function in man 80
— and **Winer, Bertram M.** The effect of splanchnicectomy on the renal excretion of electrolytes in dogs *589*
Mendeloff, Albert I. See Shank, Mendeloff, and Karl *603*
Meroney, W. H. See Darragh, Womersley, and Meroney *562*
—. See Darragh, Womersley, and Meroney 1214
Merrill, Arthur J., and **Mitchell, George L., Jr.** Continuous ACTH therapy of nephrotic syndrome in children *589*
—. See Fitzhugh, McWhorter, Estes, Warren, and Merrill 1163
Merrill, John P. See Finkenstaedt, O'Meara, and Merrill 209
—. See Finkenstaedt, O'Meara, Weller, and Merrill *567*
—. See Weller, Swan, and Merrill 729
Metcalfe, James, Romney, Seymour L., Ramsey, Lloyd H., and **Reid, Duncan E.** An approach to the measurement of uterine blood flow in pregnancy *589*
Metcoff, Jack, James, J. A., and **Gordillo, G.** Observations on renal cellular electrolyte transport systems in normal and nephrotic children *590*
Middlebrook, G., Ozerengin, H., and **Oestreicher, R.** Laboratory and clinical studies of a hemolytic test for tuberculosis *590*
Miller, George E., and **Townsend, Charles E.** The hepatic inactivation of antidiuretic hormone *590*
Miller, Joseph M., Kibrick, Sidney, and **Massell, Benedict F.** Antibody response to non-streptococcal antigens as related to rheumatic fever susceptibility 691
Miller, Max, Craig, James W., and **Woodward, Hiram, Jr.** The effect of carbohydrate deprivation on the metabolism of fructose and glucose in normal human subjects *591*
Miller, Patricia. See Petrakis, Masouredis, and Miller 952
Mills, Lewis C. See Moyer and Mills 172
Mitchell, George L. See Merrill and Mitchell *589*
Michtom, Robert J. See Dresdale, Michtom, and Schultz *563*
Mohler, William C. See Knowlton, Loeb, Stoerk, and Mohler 1175
Mollison, P. L. See Chaplin, Mollison, and Vetter 1309
Montgomery, Hugh. See Williams, Montgomery, and Horwitz 1097

Moore, Francis. See WILSON, BROOKS, and MOORE *612*
Morse, Minerva, and **Cassels, Donald E.** Arterial blood gases and acid-base balance in cyanotic congenital heart disease 837
—. See CASSELS and MORSE 52
—. See CASSELS and MORSE 824
Moses, John. See CHODOS and MOSES *560*
Motulsky, Arno G., and **Bruton, Ogden.** Hereditary nonspherocytic hemolytic disease *591*
Moyer, John H., and **Mills, Lewis C.** Hexamethonium —its effect on glomerular filtration rate, maximal tubular function, and renal excretion of electrolytes 172
—. See FORD, MOYER, and SPURR 1133
Mudge, G. H., Stanbury, S. W., and **Neuberg, H. W.** Potassium metabolism of liver mitochondria *591*
Murphy, M. Lois. See BURCHENAL, MURPHY, ELLISON, and KARNOFSKY *557*
Myers, J. D. See HAMRICK and MYERS *573*
—. See WERK, HAMRICK, MYERS, and ENGEL *610*

N

Nadell, Judith. The effects of the carbonic anhydrase inhibitor "6063" on electrolyte and acid-base balance in two normal subjects and two patients with respiratory acidosis *592*
—. The effects of the carbonic anhydrase inhibitor "6063" on electrolytes and acid-base balance in two normal subjects and two patients with respiratory acidosis 622
Naegele, Charles F. See ST. GEORGE, NAEGELE, FRENCH, ROSENMAN, and FRIEDMAN 1222
Nardi, George L. The escape of "essential" and "non essential" amino acids in the urine of severely burned patients *592*
Nelson, Don H. See BLISS, SANDBERG, NELSON, and EIK-NES 818
Nelson, Robert A., Jr. The "adhesion-disappearance" phenomenon: A specific in vitro immunologic reaction between treponema pallidum, antibody, and normal blood cells *592*
Nelson, William P., III. See ROSENBAUM, NELSON, STRAUSS, DAVIS, and ROSSMEISL 394
Nesbitt, Samuel. See AMATUZIO, STUTZMAN, VANDERBILT, and NESBITT 428
Neuberg, H. W. See MUDGE, STANBURY, and NEUBERG *591*
Neustadt, David. See STEINBROCKER, ISENBERG, SILVER, NEUSTADT, KUHN, and SCHITTONE 1045
Newerly, Katharina N. See BERSON, YALOW, POST, WISHAM, NEWERLY, VILLAZON, and VAZQUEZ *22*
Newman, E. V. See PEARCE and NEWMAN *594*
Nickel, James F., Lowrance, Preston B., Leifer, Edgar, and **Bradley, Stanley E.** Renal function, electrolyte excretion and body fluids in patients with chronic renal insufficiency before and after sodium deprivation 68
—. See LATHEM, ROOF, and NICKEL *582*
Nichols, George, Jr., and **Nichols, Nancy.** Electrolyte equilibria in erythrocytes during diabetic acidosis 113
—, —, **Weil, W. B.,** and **Wallace, W. M.** The direct measurement of the extracellular phase of tissues 1299
Nichols, Nancy. See NICHOLS and NICHOLS 113
—. See NICHOLS, NICHOLS, WEIL, and WALLACE 1299
Noll, Joan W. See FALOON and NOLL 565
Norberg, Bo. See JAMES, ABBOT, NORBERG, BIRKELAND, and EVANS *578*
Norman, Leona R. See ZOLL, NORMAN, and LINENTHAL *614*
Novack, Paul, Shenkin, Henry A., Bortin, Leonard, Goluboff, Bernard, and **Soffe, Alvin M.** The effects of carbon dioxide inhalation upon the cerebral blood flow and cerebral oxygen consumption in vascular disease 696
—. See SHENKIN, NOVAK, GOLUBOFF, SOFFE, and BORTIN 459
Nye, Robert E., Jr. See YU, LOVEJOY, JOOS, NYE, and MCCANN 130
—. See LOVEJOY, YU, NYE, JOOS, and SIMPSON *585*

O

O'Brien, William A., Jr. See SWISHER, O'BRIEN, and YOUNG *607*
Ochs, Lamar. See SENSENBACH, MADISON, and OCHS 226
—. See SENSENBACH, MADISON, and OCHS 372
Oestreicher, R. See MIDDLEBROOK, OZERENGIN and OESTREICHER *590*
Oighenstein, S. See ASSALI, KAPLAN, OIGHENSTEIN, and SUYEMOTO 922
Olson, Robert E., and **Dinning, James S.** Creatinuria in patients with leukemia and lymphoma *592*
O'Meara, Maurice P. See FINKENSTAEDT, O'MEARA, and MERRILL 209
—. See FINKENSTAEDT, O'MEARA, WELLER, and MERRILL *567*
Orloff, Jack, Kennedy, Thomas J., Jr., and **Berliner, Robert W.** The effect of potassium in nephrectomized rats with hypokalemic alkalosis 538
Ozerengin, H. See MIDDLEBROOK, OZERENGIN, and OESTREICHER *590*

P

Paine, Thomas Fite, Jr. The mode of action of streptomycin in inhibiting the respiration of escherichia coli *593*
Pallotta, Arthur. See KYLE, JEGHERS, WALSH, DOOLAN, WISHINSKY, and PALLOTTA 364
Pariser, Sanford. See KURNICK, SCHWARTZ, PARISER, and LEE 193
Park, C. R. The action of insulin on glucose uptake by muscle *593*
Parker, R. T., Fitzgerald, J. C., Perry, H. D., and **Woodward, T. E.** Treatment of typhoid carriers with penicillin and benemid *593*
Partenope, Edward A. See FREIS, ROSE, FINNERTY, and PARTENOPE *569*
—. See ROSE, HUFNAGEL, HARVEY, and PARTENOPE *598*
—. See FREIS, ROSE, PARTENOPE, HIGGINS, KELLEY, SCHNAPER, and JOHNSON 1285
Paterson, Philip Y., and **Wisseman, Charles L., Jr.** The physiologic effect of rickettsia mooseri toxin in rabbits and rats *594*
Paton, A. See REYNOLDS, PATON, FREEMAN, HOWARD, and SHERLOCK 793
Patrick, D. R. See SHETLAR, PAYNE, BULLOCK, PATRICK, HELLBAUM, and ISHMAEL 1208
Patterson, John L., Jr. See HEYMAN and PATTERSON *574*
Paul, William D. See HODGES, PAUL, ROUTH, DAUM, and BRADBURY *575*

Payne, R. W. See SHETLAR, PAYNE, BULLOCK, PATRICK, HELLBAUM, and ISMAEL 1208
Peacock, Wendell. See BECKER, RALL, PEACOCK, and RAWSON 149
Pearce, M. L., and **Newman, E. V.** Somé postural adjustments of salt and water excretion *594*
Pearson, Olof H., West, Charles D., and **Treves, Norman E.** The role of ovarian function in the growth of mammary carcinoma in man *594*
Perera, George A. Depressor effects of potassium-deficient diets in hypertensive man 633
Perle, G. See SLEISENGER, ALMY, GILDER, and PERLE 466
Perry, H. D. See PARKER, FITZGERALD, PERRY, and WOODWARD *593*
Perry, H. Mitchell, Jr. Chemical observations on l-hydrazinophthalazine *595*
Perry, William D. See STETSON, RAMMELKAMP, PERRY, KRAUSE, and KOHEN *606*
Petersdorf, R. G., and **Welt, L. G.** The effect of an infusion of hyperoncotic albumin on the excretion of water and solutes 283
Petrakis, N. L., Masouredis, S. P., and **Miller, Patricia.** The local blood flow in human bone marrow in leukemia and neoplastic diseases as determined by the clearance rate of radioiodide (I^{131}) 952
—. See BIERMAN, BYRON, KELLY, GILFILLAN, WHITE, FREEMAN, and PETRAKIS 637
Phillips, Gerald B. See SCHWARTZ, SEEGMILLER, PHILLIPS, GABUZDA, and DAVIDSON *602*
—. See GABUZDA, PHILLIPS, and DAVIDSON 899
Pinsky, Ruth. See WOLF and PINSKY *613*
Pirofsky, Bernard. The determination of blood viscosity in man by a method based on Poiseuille's law *292*
Pitney, Robert, Beard, Marion, and **Sanneman, Everett.** In vitro binding capacity of human serum for vitamin B_{12} *595*
Plamondon, Charles A. See ASPER, SELENKOW, and PLAMONDON *552*
Plitman, Gerald. See STEFANINI and PLITMAN *606*
Popper, Hans. See DE LA HUERGA, GYÖRGY, WALDSTEIN, KATZ, and POPPER 1117
Post, Joseph. See BERSON, YALOW, POST, WISHAM, NEWERLY, VILLAZON, and VAZQUEZ 22
—. See BERSON, YALOW, SCHREIBER, and POST *554*
—. See BERSON, YALOW, SCHREIBER, and POST 746
Post, Robert S. See EPSTEIN, POST, and MCDOWELL 233
Prinzmetal, Myron, Kennamer, S. Rexford, Shaw, Clinton, Maxwell, Morton, and **Bernstein, Jack.** The relationship between pathologic and electrocardiographic changes in myocardial disease *595*

Q

Querido, A. See VAN BEKKUM and QUERIDO 1061
Quinn, Murray. See KLEEMAN, BASS, and QUINN 736

R

Rabinowitz, Murray, and **McDonald, Lawson.** The clinical and physiological contrasts between mitral stenosis and mitral insufficiency *595*
—. See MCDONALD and RABINOWITZ *588*
Ragan, Charles. See JESSAR, GANZELL, and RAGAN 480
Rall, J. E., Robbins, Jacob, Becker, David, and **Rawson, Rulon W.** The metabolism of labeled l-tri-iodothyronine, l-thyroxine and d-thyroxine *596*
—. See BECKER, RALL, PEACOCK, and RAWSON 149
Rammelkamp, Charles H., Jr., and **Weaver, Robert S.** Acute glomerulonephritis. The significance of the variations in the incidence of the disease 345
—. See STETSON, RAMMELKAMP, PERRY, KRAUSE, and KOHEN *606*
Ramsey, Lloyd H. See METCALFE, ROMNEY, RAMSEY, and REID *589*
Randall, Henry T. See ROBERTS, RANDALL, and FARR *597*
Ranney, Helen M., Larson, Daniel L., and **McCormack, George H., Jr.** Some clinical, biochemical and genetic observations on hemoglobin C. 1277
—. See LARSON and RANNEY 1070
Rantz, Lowell A. Serum hemolysin for erythrocytes coated with non-species specific bacterial products in health and in various diseases *596*
Ratnoff, Oscar D. Studies on a proteolytic enzyme in human plasma. IX. Fibrinogen and fibrin as substrates for the proteolytic enzyme of plasma 473
—. Delayed blood coagulation in multiple myeloma *596*
Rawson, Rulon W. See BECKER, RALL, PEACOCK, and RAWSON 149
—. See RALL, ROBBINS, BECKER, and RAWSON *596*
Rehm, Warren S. See BRODSKY, REHM, and MCINTOSH *556*
Reid, Duncan E. See METCALFE, ROMNEY, RAMSEY, and REID *589*
Reid, Virginia. See CARREREA, REID, and KURNICK *559*
Reifenstein, Robert W. See CHALMERS, ECKHARDT, REYNOLDS, CIGARROA, DEANE, REIFENSTEIN, and SMITH *559*
Reiser, Morton F. See BRUST, HAGANS, and REISER *556*
Relman, Arnold S., Roy, Arlene M., and **Schwartz, William B.** Effect of rubidium on acid-base balance in potassium deficient and normal rats *597*
—, **Etsten, Benjamin,** and **Schwartz, William B.** The regulation of renal bicarbonate reabsorption by plasma carbon dioxide tension 972
—. See SCHWARTZ and RELMAN 258
—. See SCHWARTZ, JENSON, and RELMAN *602*
Reynolds, T. B., Paton, A., Freeman, M., Howard, F., and **Sherlock, Sheila.** The effect of hexamethonium bromide on splanchnic blood flow, oxygen consumption and glucose output in man 793
Reynolds, William E. See CHALMERS, ECKHARDT, REYNOLDS, CIGARROA, DEANE, REIFENSTEIN, and SMITH *559*
Rice, Marion L., and **Hughes, Felix.** Histopathology and bacteriology of biopsied parietal pleura *597*
Rich, M. See SCHEINBERG, BLACKBURN, and RICH 125
—. See SCHEINBERG, BLACKBURN, SASLAW, RICH, and BAUM 720
Richards, Anne. See TURNER, MCCORMACK, and RICHARDS 801
Richards, Dickinson W., Jr. See CATHCART, FIELD, and RICHARDS 5
Rifkin, Harold. See BERKMAN, RIFKIN, and ROSS. 415
Riley, Richard L. See CARROLL, COHN, and RILEY 510
Rinfret, A. P. See LIDDLE, RINFRET, ISLAND, and FORSHAM *584*

Robbins, Jacob. See RALL, ROBBINS, BECKER, and RAWSON *596*

Roberts, Kathleen E., Randall, Henry T., and **Farr, Hollon W.** Acute alterations in blood volume, plasma electrolytes and the electrocardiogram produced by oral administration of hypertonic solutions to totally gastrectomized patients *597*

Robertson, James S. See GAMBLE, ROBERTSON, HANNIGAN, FOSTER, and FARR 483

Robin, Eugene D., and **Gardner, Frank H.** Cerebral metabolism and hemodynamics in pernicious anemia *598*

Robinson, Jean. See LICHTMAN, WATSON, FELDMAN, GINSBERG, and ROBINSON 1229

Rodbard, Simon. The utility of the Korotkoff sounds in the assessment of cardiovascular function *598*

Rogers, Walter F., Jr., Burdick, Mary P., and **Burnett, George R.** The effect of antibiotics on the excretion of urinary phenols *598*

Romney, Seymour L. See METCALFE, ROMNEY, RAMSEY, and REID *589*

Roof, Betty S. See LATHEM, ROOF, and NICKEL *582*

Rose, John C., Hufnagel, Charles A., Harvey, W. Proctor, and **Partenope, Edward A.** Hemodynamic alterations produced by a plastic valvular prosthesis for severe aortic insufficiency in man *598*

—. See FREIS, ROSE, FINNERTY, and PARTENOPE *569*

—. See FREIS, ROSE, PARTENOPE, HIGGINS, KELLEY, SCHNAPER, and JOHNSON 1285

Rosecan, Marvin, and **Daughaday, William H.** A comparison of insulin treatment with and without added carbohydrate in human diabetic ketosis *599*

Rosen, Hyman. See LEVENSON, ADAMS, ROSEN, and TAYLOR 497

Rosenberg, I. N. Metabolic effects of an anterior pituitary extract *599*

Rosenbaum, Jack D., Nelson, William P., III, Strauss, Maurice B., Davis, Robert K., and **Rossmeisl, Elsie C.** Variation in the diuretic response to ingested water related to the renal excretion of solutes 394

Rosenfeld, Robert. See HELLMAN, ROSENFELD FUKUSHIMA, EIDINOFF, BRUNSCHWIG, and GALLAGHER *573*

Rosenman, Ray H., Friedman, Meyer, and **Byers, Sanford O.** Observations concerning the cholate: cholesterol relationship in clinical and experimental nephrosis 121

—. See ST. GEORGE, NAEGELE, FRENCH, ROSENMAN, and FRIEDMAN 1222

Rosenthal, Nathan. See ROSENTHAL, DRESKIN, and ROSENTHAL *599*

Rosenthal, Otto. See HILLS, CHALMERS, WEBSTER, and ROSENTHAL 1236

Rosenthal, Robert L., Dreskin, O. Herman, and **Rosenthal, Nathan.** Studies on hemorrhagic diseases caused by deficiencies of plasma thromboplastin factors *599*

Ross, George. See BERKMAN, RIFKIN, and ROSS 415

—. See BERNSTEIN, WESTON, ROSS, GROSSMAN, HANENSON, and LEITER 422

Ross, Joseph F. See EBAUGH, ROSS, and EMERSON *563*

—. See FINCH, ROSS, and EBAUGH *567*

—. See EBAUGH, EMERSON, and ROSS 1260

Rossmeisl, Elsie C. See ROSENBAUM, NELSON, STRAUSS, DAVIS, and ROSSMEISL 394

Rottier, Paul B. The erythematogenous action of ultraviolet light on human skin. I. Some measurements of the spectral response with continuous and intermittent light 681

Routh, Joseph I. See HODGES, PAUL, ROUTH, DAUM, and BRADBURY *575*

Rowe, George G. See CROSLEY, ROWE, MCKENNA, and CRUMPTON *561*

Roy, Arlene M. See RELMAN, ROY, and SCHWARTZ *597*

Rubin, C. E., Massey, B. W., Stonecypher, D. D., and **Dobson, R. L.** New cytologic techniques for in vivo study of the pathogenesis of gastrointestinal neoplasia *600*

Rubin, George. See WHITE, KURTZ, and RUBIN *611*

—. See WHITE, RUBIN, and LEITER 931

Rubini, M. See LIPSKY, ALPER, RUBINI, GORDON, and VAN ECK *584*

Rundles, R. W., Arends, Tulio, and **Coonrad, Evelyn V.** Anomalous serum proteins in malignant lymphomas and leukemia *600*

Russ, Ella M. See EDER and RUSS *564*

Rutstein, David D., Ingenito, Estelle F., and **Bourneuf, Jeanne M.** The determination of albumin in human serum. A method based on the interaction of albumin with an anionic dye-2-(4'-hydroxybenzeneazo) benzoic acid *600*

S

Saltzman, Abraham, and **Caraway, Wendell T.** Cinnamic acid as a test substance in the evaluation of liver function 711

Samet, Philip. See FERRER, HARVEY, SAMET, and COURNAND *566*

Sandberg, Avery A. See TYLER, SANDBERG, and EIK-NES 608

—. See BLISS, SANDBERG, NELSON, and EIK-NES 818

Sanneman, Everett. See PITNEY, BEARD, and SANNEMAN *595*

Santos, Roberto F. See LEAF, BARTTER, SANTOS, and WRONG *582*

—. See LEAF, BARTTER, SANTOS, and WRONG 868

Saslaw, M. See SCHEINBERG, BLACKBURN, SASLAW, RICH, and BAUM 720

Sborov, Victor M., Giges, Burton, and **Mann, Joseph.** The incidence of hepatitis in Korean casualties following the use of pooled plasma *601*

Schaefer, Louis E. See ADLERSBERG, WANG, and SCHAEFER *550*

Scheinberg, P., Blackburn, I., and **Rich, M.** The effects of intravenous priscoline on cerebral circulation and metabolism 125

—, —, **Saslaw, M., Rich, M.,** and **Baum, G.** Cerebral circulation and metabolism in pulmonary emphysema and fibrosis with observations on the effects of mild exercise 720

Schittone, Marilyn. See STEINBROCKER, ISENBERG, SILVER, NEUSTADT, KUHN, and SCHITTONE 1045

Schieve, James F., and **Wilson, William P.** The changes in cerebral vascular resistance of man in experimental alkalosis and acidosis 33

Schiff, Leon. See BILLING, CONLON, HEIN, and SCHIFF 214

Schmidt, Carl F. See CONN, WOOD, and SCHMIDT 1180

Schnaper, Harold W. See FREIS, ROSE, PARTENOPE, HIGGINS, KELLEY, SCHNAPER, and JOHNSON 1285

Schoenberger, James A., Kark, Robert M., Kroll, George, and **Eckert, Edward.** Endothelial permeability in man studied with albumin-I^{131} injected intravenously or intraperitoneally *601*

Schottstaedt, William W., and **Hinkle, Lawrence E., Jr.** Renal retention and diuresis occurring in association with certain life situations *602*

Schreiber, Sidney S. See BERSON, YALOW, SCHREIBER, and POST *554*

—. See BERSON, YALOW, SCHREIBER, and POST 746

Schreiner, George E. See FREINKEL, SCHREINER, and ATHENS 138

Schultz, Alvin L., Hammarsten, James F., Heller, Ben I., and **Ebert, Richard V.** A critical comparison of the T-1824 dye and iodinated albumin methods for plasma volume measurement 107

Schultz, Martin. See DRESDALE, MICHTOM, and SCHULTZ *563*

Schwarz, Haiyim. See LONDON and SCHWARZ 1248

Schwartz, Irving L., Thaysen, Jorn Hess, and **Dole, Vincent P.** The clearance of urea by the sweat glands *602*

—. See DOLE, DAHL, SCHWARTZ, COTZIAS, THAYSEN, and HARRIS 185

Schwartz, Lawrence I. See KURNICK, SCHWARTZ, PARISER, and LEE 193

Schwartz, R., Seegmiller, J. E., Phillips, G. B., Gabuzda, G. J., Jr., and **Davidson, C. S.** Electrolytes, ammonia and glutamine in hepatic coma *602*

Schwartz, William B., and **Relman, Arnold S.** Metabolic and renal studies in chronic potassium depletion resulting from overuse of laxatives *258*

—, **Jenson, Robert L.,** and **Relman, Arnold S.** Renal and extrarenal adjustments to acidifying salts in sodium-depleted subjects *602*

—. See D'AGOSTINO, LEADBETTER, and SCHWARTZ 444

—. See RELMAN, ROY, and SCHWARTZ *597*

—. See RELMAN, ETSTEN, and SCHWARTZ 972

Scialabba, Dominick. See ZIFF, WILSON, SCIALABBA, SCULL, and GLYN *614*

Scott, James L., Jr. See BURNS, ENGEL, VIAU, SCOTT, HOLLINGSWORTH, and WERK 781

Scull, Edward. See ZIFF, WILSON, SCIALABBA, SCULL, and GLYN *614*

Seegal, Beatrice C. See WERTHEIM, LYTTLE, LOEB, EARLE, SEEGAL, and SEEGAL 359

Seegal, David. See WERTHEIM, LYTTLE, LOEB, EARLE, SEEGAL, and SEEGAL 359

Seegmiller, J. E. See SCHWARTZ, SEEGMILLER, PHILLIPS, GABUZDA, and DAVIDSON *602*

Seldin, Donald W. See GOODYER and SELDIN 242

—. See WALSER, SELDIN, and GROLLMAN *299*

—. See GOODYER and SELDIN *570*

Selenkow, Herbert A. See ASPER, SELENKOW, and PLAMONDON *552*

Seligson, David. See SMITH, ETTINGER, and SELIGSON *273*

Sensenbach, Willis, Madison, Leonard, and **Ochs, Lamar.** A comparison of the effects of *l*-nor-epinephrine, synthetic *l*-epinephrine and U.S.P. epinephrine upon cerebral blood flow and metabolism in man *226*

—, —, —. The effect of ACTH and cortisone on cerebral blood flow and metabolism 372

Serstock, Doris. See WISE and SERSTOCK *612*

Shadle, O. W. See EPSTEIN, SHADLE, FERGUSON, and MCDOWELL 543

Shank, Robert E., Mendeloff, Albert I., and **Karl, Michael M.** Microchemical study of constituents of liver in patients with hepatic disease *603*

Shapiro, Alvin P. See GROLLMAN and SHAPIRO 312

Shaw, Clinton. See PRINZMETAL, KENNAMER, SHAW, MAXWELL, and BERNSTEIN *595*

Sheffield, Roy S. See DAILY and SHEFFIELD *562*

Shenkin, Henry A., Novack, Paul, Goluboff, Bernard, Soffe, Alvin M., and **Bortin, Leonard.** The effects of aging, arteriosclerosis, and hypertension upon the cerebral circulation 459

—. See NOVACK, SHENKIN, BORTIN, GOLUBOFF, and SOFFE 696

Shen, Shu Chu. Hemoglobin tolerance in various types of anemia *603*

Sherry, Sol, and **Troll, Walter.** The nature of the action of thrombin. Enzymatic attack on a synthetic substrate *603*

Sherlock, Sheila. See REYNOLDS, PATON, FREEMAN, HOWARD, and SHERLOCK 793

Sherwood, Helen K. See WILLIAMS, SHERWOOD, and EINHEBER *612*

Shetlar, M. R., Payne, R. W., Bullock, Jane A., Patrick, D. R., Hellbaum, Arthur A., and **Ishmael, William K.** Comparative studies of serum polysaccharides in rheumatoid arthritis and degenerative joint disease 1208

Shipley, E. S. See CHART and SHIPLEY *560*

Shulman, N. Raphael. The relationship between fibrinolysis and coagulation defects *604*

Siegel, A. See BING, SIEGEL, VITALE, BALBONI, and SPARKS *556*

Siegel, Alan C. See BROCK and SIEGEL 630

Silliman, Joan. See ANDERSON, WILLCOX, SILLIMAN, and BLOUNT 490

Silver, Murray. See STEINBROCKER, ISENBERG, SILVER, NEUSTADT, KUHN, and SCHITTONE 1045

Simeone, F. A. See MCARTHUR, SMART, HARTING, GAUTIER, GODLEY, SWALLOW, SIMEONE, and HUME *587*

Simpson, John H. See LOVEJOY, YU, NYE, JOOS, and SIMPSON *585*

Singer, R. B., Elkinton, J. R., Barker, E. S., and **Clark, J. K.** Transfers of cellular cations during acute respiratory alkalosis and acidosis experimentally produced in man *604*

Sinton, David W. See LEWIS, SINTON, and KNOTT *583*

Sirota, Jonas H., and **Yü, T. F.** Effects of phenylbutazone (3,5-dioxo-1, 2-diphenyl-4-n-butapyrazolidine) on urate clearance and other discrete renal functions in gout *605*

—. See YÜ, SIROTA, and GUTMAN 1121

Slater, Robert J. See EISENMENGER and SLATER *564*

Sleisenger, M. H., Almy, T. P., Gilder, H., and **Perle, G.** Colorimetric determination of serum cholinesterase: Its value in hepatic and biliary tract diseases 466

Slobody, Lawrence B. See LANGE, SLOBODY, and STRANG *581*

Smart, G. A. See MCARTHUR, SMART, HARTING, GAUTIER, GODLEY, SWALLOW, SIMEONE, and HUME *587*

Smith, Alfred. See ZIFF, KANTOR, BIEN, and SMITH 1253

Smith, Clifford W. See CHALMERS, ECKHARDT, REYNOLDS, CIGARROA, DEANE, REIFENSTEIN, and SMITH *559*

Smith, Lloyd H., Jr., Ettinger, Richard H., and **Seligson, David.** A comparison of the metabolism of fructose and glucose in hepatic disease and diabetes mellitus 273

Smythe, C. M., Gilmore, J. P., and **Handford, S. W.** The effect of l-nor-epinephrine on estimated hepatic blood flow in the normal anesthetized dog *605*

—. See BRADLEY, SMYTHE, FITZPATRICK, and BLAKEMORE 526

Soberman, Robert J. See FARBER and SOBERMAN *566*

Soffe, Alvin M. See SHENKIN, NOVACK, GOLUBOFF, SOFFE, and BORTIN 459

—. See NOVACK, SHENKIN, BORTIN, GOLUBOFF, and SOFFE 696

Sokoloff, Louis, Wechsler, Richard L., Mangold, Renward, Balls, Kent, and **Kety, Seymour S.** Cerebral blood flow and oxygen consumption in hyperthyroidism before and after treatment 202

Sparks, E. See BING, SIEGEL, VITALE, BALBONI, and SPARKS *556*

Spencer, Herta. See LEWIN, SPENCER, and LASZLO *584*

Sprague, Howard B. See WHEELER and SPRAGUE *611*

Spurr, Charles L. See FORD, MOYER, and SPURR 1133

Squires, Russell D., Huth, Edward J., and **Elkinton, J. Russell.** Metabolic balance studies on a patient sustaining large renal losses of potassium *605*

Stanbury, S. W. See MUDGE, STANBURY, and NEUBERG *591*

Stanley, Malcolm M. An indicator method for study of intestinal absorption in man *606*

Stefanini, Mario, and **Plitman, Gerald.** Antigenicity of platelets and evidence for platelet groups in humans *606*

Steinbrocker, Otto, Isenberg, S. Allen, Silver, Murray, Neustadt, David, Kuhn, Paul, and **Schittone, Marilyn.** Observations on pain produced by injection of hypertonic saline into muscles and other supportive tissues 1045

Steinfeld, Jesse L. See BIERMAN, CORDES, KELLY, LITTMAN, STEINFELD, and YAMAUCHI *555*

Stern, Kurt G. See MACK, WOLF, and STERN *862*

Stetson, Chandler A., Rammelkamp, Charles H., Perry, William D., Krause, Richard M., and **Kohen, Roland J.** The relationship of streptococcal infections to acute glomerulonephritis *606*

Stetten, DeWitt, Jr. See BENEDICT, YÜ, BIEN, GUTMAN, and STETTEN 775

—. See BIEN, YÜ, BENEDICT, GUTMAN, and STETTEN 778

Stewart, W. B., Vassar, Philip S., and **Stone, Robert S.** Iron absorption in dogs during anemia due to acetylphenylhydrazine 1225

St. George, Shirley, Naegele, Charles F., French, Frank S., Rosenman, Ray H., and **Friedman, Meyer.** A quantitative study of the digitoxin content of edema fluids 1222

—. See FRIEDMAN and ST. GEORGE *569*

Stoerk, Herbert C. See KNOWLTON, LOEB, STOERK, and MOHLER 1175

Stollerman, Gene H. The use of hemolysis inhibition in the study of experimental and clinical hyperlipemia *607*

Stone, Robert S. See STEWART, VASSAR, and STONE 1225

Stonecypher, D. D. See RUBIN, MASSEY, STONECYPHER, and DOBSON *600*

Strang, Ruth H. See LANGE, SLOBODY, and STRANG *581*

Strauss, Maurice B. See ROSENBAUM, NELSON, STRAUSS, DAVIS, and ROSSMEISL 394

—. See BIRCHARD and STRAUSS 807

Stutzman, Francis L. See AMATUZIO, STUTZMAN, VANDERBILT, and NESBITT 428

—. See FLINK, STUTZMAN, ANDERSON, and KONIG *568*

Stutzman, F. L. See HELLER, HAMMARSTEN, and STUTZMAN 858

Sundharagiati, Bundham, and **Wright, Claude-Starr.** A clinical and experimental study of the erythrocyte ultrastructure membrane with the electron microscope 979

Suyemoto, R. See ASSALI, KAPLAN, OIGHENSTEIN, and SUYEMOTO *922*

Swallow, K. A. See MCARTHUR, SMART, HARTING, GAUTIER, GODLEY, SWALLOW, SIMEONE, and HUME *587*

Swan, H. J. C. See WOOD and SWAN *613*

Swan, Roy C. See WELLER, SWAN, and MERRILL 729

Sweet, Avron Y. See KATCHER, LEVITT, SWEET, and HODES 1013

Swisher, Scott N., Jr., O'Brien, William A., Jr., and **Young, Lawrence E.** Studies of the mechanisms of destruction of transfused incompatible erythrocytes by isoantibodies *607*

T

Talmage, D. W. See WEINSTEIN, DERN, and TALMAGE *609*

Tarail, Robert, and **Logsdon, George R.** The response of the kidney and body fluids to hypertonic saline in normal subjects and in patients with diabetes insipidus *607*

Tateno, Isao. See KILBOURNE and TATENO *580*

Taylor, F. H. Laskey. See LEVENSON, ADAMS, ROSEN, and TAYLOR 497

Thaysen, Jørn Hess. See DOLE, DAHL, SCHWARTZ, COTZIAS, THAYSEN, and HARRIS 185

—. See SCHWARTZ, THAYSEN, and DOLE *602*

Theilen, Ernest O. See BEDELL, CULBERTSON, EHRENFAFT, AMES, and THEILEN *554*

Thomas, Lewis. See DENNY, GOOD, and THOMAS *562*

—. See DENNY and THOMAS 1085

Thorup, Oscar A. See WOMERSLEY, THORUP, and WELT *613*

Tinsley, Clarence M. See WILKINS, TINSLEY, CULBERTSON, BURROWS, JUDSON, and BURNETT 1101

Tobian, Louis, Jr., and **Binion, John.** Tissue electrolytes in renal and DCA hypertension *608*

Tokuyama, Ichiro. See MADDOCK, TOKUYAMA, and LEACH *586*

Tolbert, Bert M. See BERLIN, TOLBERT, and LEE 1

Tomlinson, Lois J. See LARSON and TOMLINSON 317

Tosteson, D. C. Potassium exchange in sickle cell anemia red cells *608*

Townsend, Charles E. See MILLER and TOWNSEND *590*

Traeger, Cornelius H. See BATTERMAN and TRAEGER *553*

Treves, Norman E. See Pearson, West, and Treves *594*
Troll, Walter. See Sherry and Troll *603*
Turner, Kenneth B., McCormack, George H., Jr., and **Richards, Anne.** The cholesterol-esterifying enzyme of human serum. I. In liver disease 801
Turner, Louis B. See Levitt and Turner *583*
Tyler, Frank H., Sandberg, Avery A., and **Eik-Nes, Kris.** Adrenal cortical reserve and its relation to stress *608*

V

Valentine, William N., Follette, James H., and **Lawrence, John S.** The glycogen content of human leukocytes in health and in various disease states 251
Van Bekkum, D. W., and **Querido, A.** Inorganic phosphate response to intravenous glucose administration in progressive muscular dystrophy 1061
Vanderbilt, Muriel J. See Amatuzio, Stutzman, Vanderbilt, and Nesbitt 428
VanderLaan, W. P. Direct effects of the anterior pituitary on the ventral prostate in rats *609*
Van Eck. See Lipsky, Alper, Rubini, Gordon, and Van Eck *584*
Vassar, Philip S. See Stewart, Vassar, and Stone 1225
Vazquez, Oscar N. See Berson, Yalow, Post, Wisham, Newerly, Villazon, and Vazquez 22
Vetter, H. See Chaplin, Mollison, and Vetter 1309
Viau, Alberto. See Burns, Engel, Viau, Scott, Hollingsworth, and Werk 781
Villazon, Manuel J. See Berson, Yalow, Post, Wisham, Newerly, Villazon, and Vazquez 22
Vitale, A. See Bing, Siegel, Vitale, Balboni, and Sparks *556*
Von Euler, Ulf S. See Luft and Von Euler 1065

W

Waldstein, Sheldon S. See Ettinger, Giges, and Waldstein *565*
Waldstein, Sheldon. See De la Huerga, György, Waldstein, Katz, and Popper 1117
Wallace, W. M. See Nichols, Nichols, Weil, and Wallace 1299
Walser, Mackenzie, Seldin, Donald W., and **Grollman, Arthur.** An evaluation of radiosulfate for the determination of the volume of extracellular fluid in man and dogs 299
Walsh, William P. See Kyle, Jeghers, Walsh, Doolan, Wishinsky, and Pallotta 364
Wang, Chun-I. See Adlersberg, Wang, and Schaefer *550*
Ware, Arnold G. See Lanchantin and Ware 381
Warren, J. V. See McIntosh, Burnum, Hickam, and Warren *588*
Warren, James V. See Fitzhugh, McWhorter, Estes, Warren, and Merrill 1163
Webster, G. D., Jr. See Hills, Chalmers, Webster, and Rosenthal 1236
Waterhouse, Christine, Keu'mann, E. Henry, and **Fenninger, Leonard D.** The use of modified globin as a protein supplement in normal individuals 964
Wathen, Joan D. See Best, Wathen, and Brodsky *555*
Watson, R. Janet. See Lichtman, Watson, Feldman, Ginsberg, and Robinson 1229
Weaver, Robert S. See Rammelkamp and Weaver 345
Webster, George D. See Hills, Chalmer, and Webster *574*
Wechsler, Richard L. See Sokoloff, Wechsler, Mangold, Balls, and Kety 202
Weigand, F. A. See Greenman, Weigand, and Danowski *572*
Weil, W. B. See Nichols, Nichols, Weil, and Wallace 1299
Weinstein, I. M., Dern, R. J., and **Talmage, D. W.** Studies on the mechanism of hemolysis in primaquine-sensitive Negroes *609*
Weir, David R. The use of calcium pantothenate for myasthenia gravis *609*
Weisberger, Austin S., and **Levine, Bennett.** Utilization of S_{35} labelled L-Cystine by normal and leukemic leukocytes *610*
Weisman, Russell, Jr. See Hurley and Weisman *576*
Weller, John M., Swan, Roy C., and **Merrill, John P.** Changes in acid-base balance of uremic patients during hemodialysis 729
—. See Finkenstaedt, O'Meara, Weller, and Merrill *567*
Welt, L. G. See Petersdorf and Welt 283
Welt, Louis G. See Womersley, Thorup, and Welt *613*
Welton, Ted L. See Culbertson, Easton, and Welton *561*
Werk, Emile E., Jr., Hamrick, Ladd W., Jr., Myers, J. D., and **Engel, Frank L.** Splanchnic ketone production before and during the intravenous infusion of sodium octanoate *610*
Werk, Emile. See Burns, Engel, Viau, Scott, Hollingsworth, and Werk 781
Werkö, Lars. See Josephson, Ek, Bucht, Werkö, Kallas, and Grieg 1184
Wertheim, Arthur, Lyttle, John D., Loeb, Emily N., Earle, David P., Jr., Seegal, Beatrice C., and **Seegal, David.** The association of type-specific hemolytic streptococci with acute glomerulonephritis at the Presbyterian and Babies Hospitals, New York, N. Y. 1936–1942 359
Wessler, Stanford. Experimentally produced phlebothrombosis in the study of thromboembolism *610*
—. Studies in intravascular coagulation. II. A comparison of the effect of dicumarol and heparin on clot formation in isolated venous segments 650
West, Charles D. See Pearson, West, and Treves *594*
Weston, Raymond E., Hanenson, Irwin B., Grossman, Jacob, Berdasco, Gloria A., and **Wolfman, Morris.** Natriuresis and chloruresis following pitressin-induced water retention in non-edematous patients: Evidence of a homeostatic mechanism regulating body fluid volume *611*
—. See Grossman, Weston, and Leiter 161
—. See Bernstein, Weston, Ross, Grossman, Hanenson, and Leiter 422
Wheeler, Edwin O., and **Sprague, Howard B.** The prevalence and significance of hypercholesterolemia among children and siblings of patients with hypercholesterolemic xanthomatosis *611*
White, Abraham G., Kurtz, Martin, and **Rubin, George.** Impaired renal response to the antidiuretic hormone in chronic renal disease *611*

—, **Rubin, George,** and **Leiter, Louis.** Studies in edema. IV. Water retention and the antidiuretic hormone in hepatic and cardiac disease 931
White, L. P. See BIERMAN, BYRON, KELLY, GILFILLAN, WHITE, FREEMAN, and PETRAKIS 637
Whittenberger, James L. See MEAD and WHITTENBERGER *588*
Wightman, K. J. R. See BARDAWILL, BRITTON, and WIGHTMAN *553*
Wilkins, Robert W., Tinsley, Clarence M., Culbertson, James W., Burrows, Belton A., Judson, Walter E., and **Burnett, Charles H.** The effects of venous congestion of the limbs upon renal clearances and the excretion of water and salt. I. Studies in normal subjects and in hypertensive patients before and after splanchnicectomy 1101
Willcox, Mary L. See ANDERSON, WILLCOX, SILLIMAN, and BLOUNT 490
Williams, Arnold H., Sherwood, Helen K., and **Einheber, Albert.** Blood flow and transcapillary exchange in hemorrhagic shock *612*
Williams, M. Henry, Jr. Pulmonary function in Boeck's sarcoid 909
—. Pulmonary function studies in mitral stenosis before and after commissurotomy 1094
Williams, Phyllis G., Montgomery, Hugh, and **Horwitz, Orville.** Oxygen tension of tissues by the polarographic method. VI. Effect of changes in position on oxygen tension of the skin of toes 1097
Wilson, Graham, Brooks, Laura, and **Moore, Francis.** Sodium maldistribution in acute and chronic disease *612*
Wilson, Hildegard. See ZIFF, WILSON, SCIALABBA, SCULL, and GLYN *614*
Wilson, William P. See SCHIEVE and WILSON 33
Winer, Bertram M. See MEILMAN and WINER *589*
Wintrobe, M. M. See LAHEY, GUBLER, CARTWRIGHT, and WINTROBE 322
—. See LAHEY, GUBLER, CARTWRIGHT, and WINTROBE 329
—. See GUBLER, LAHEY, CARTWRIGHT, and WINTROBE 405
Wise, Robert I., and **Serstock, Doris.** Experimental and clinical studies on small-colony (G) variants of micrococcus pyogenes obtained with antibiotics *612*
Wisham, Lawrence H. See BERSON, YALOW, POST, WISHAM, NEWERLY, VILLAZON, and VAZQUEZ 22
Wisseman, Charles L., Jr. See PATERSON and WISSEMAN *594*
Wishinsky, Henry. See KYLE, JEGHERS, WALSH, DOOLAN, WISHINSKY, and PALLOTTA 364
Witkin, Lloyd. See FINNERTY, WITKIN, and FAZEKAS *568*
Wolf, Stewart, and **Pinsky, Ruth.** Toxic effects following placebo administration *613*
—. See MACK, WOLF, and STERN 862
Wolfman, Morris. See WESTON, HANENSON, GROSSMAN, BERDASCO, and WOLFMAN *611*
Wolsky, Leonard. See HAMOLSKY, FREEDBERG, KURLAND, and WOLSKY 453
Wolter, Janet. See ARMSTRONG, MCLEOD, WOLTER, and KUKRAL *552*
Womersley, R. A. See DARRAGH, WOMERSLEY, and MERONEY *562*
—. See DARRAGH, WOMERSLEY, and MERONEY 1214
Womersley, Richard, Thorup, Oscar A., and **Welt, Louis G.** The influence of enhanced sodium reabsorption by DOCA and compounds E and F on the rates of excretion of water, potassium and ammonia *613*
Wood, E. H., and **Swan, H. J. C.** Localization of cardiac defects by dilution curves recorded following injection of T-1824 at multiple sites in the heart and great vessels during cardiac catheterization *613*
Wood, John C. See CONN, WOOD, and SCHMIDT 1180
Woodward, Hiram, Jr. See MILLER, CRAIG, and WOODWARD *591*
Woodward, T. E. See PARKER, FITZGERALD, PERRY, and WOODWARD *593*
Wright, Claude-Starr. See SUNDHARAGIATI and WRIGHT 979
Wrong, Oliver. See LEAF, BARTTER, SANTOS, and WRONG *582*
—. See LEAF, BARTTER, SANTOS, and WRONG 868

Y

Yalow, Rosalyn S. See BERSON, YALOW, POST, WISHAM, NEWERLY, VILLAZON, and VAZQUEZ 22
—. See BERSON, YALOW, SCHREIBER, and POST *554*
—. See BERSON, YALOW, SCHREIBER, and POST 746
Yamauchi, Paul K. See BIERMAN, CORDES, KELLY, LITTMAN, STEINFELD, and YAMAUCHI *555*
Young, Lawrence E. See SWISHER, O'BRIEN, and YOUNG *607*
Yu, Paul N. G., Lovejoy, Frank W., Jr., Joos, Howard A., Nye, Robert E., Jr., and **McCann, William S.** Studies of pulmonary hypertension. I. Pulmonary circulatory dynamics in patients with pulmonary emphysema at rest 130
—. See LOVEJOY, YU, NYE, JOOS, and SIMPSON *585*
Yü, Tsai Fan, Sirota, Jonas H., and **Gutman, Alexander B.** Effect of phenylbutazone (3,5 dioxo-1,2-Diphenyl-4-n-Butylpyrazolidine) on renal clearance of urate and other discrete renal functions in gouty subjects 1121
—. See SIROTA and YÜ *605*
—. See BENEDICT, YÜ, BIEN, GUTMAN, and STETTEN 775
—. See BIEN, YÜ, BENEDICT, GUTMAN, and STETTEN 778

Z

Zarafonetis, Chris J. D., and **Colville, Virginia F.** The heterophile agglutination test and its interpretation *614*
Ziff, Morris, Wilson, Hildegard, Scialabba, Dominick, Scull, Edward, and **Glyn, John.** Rate of disappearance and metabolism of hydrocortisone and cortisone in the synovial cavity *614*
—, **Kantor, Thomas, Bien, Edward,** and **Smith, Alfred.** Studies on the composition of the fibrinoid material of the subcutaneous nodule of rheumatoid arthritis 1253
—. See DEANE, ZIFF, and BUNIM 879
Zinn, Willard J. See FIELD, ZINN, and GRIFFITH *566*
Zoll, Paul M., Norman, Leona R., and **Linenthal, Arthur J.** Control of cardiac rhythm by an external electric pacemaker *614*

SUBJECT INDEX

(Page numbers in italics refer to abstracts)

A

Abscess, pyogenic, blood copper in, 332
Acetylphenylhydrazine, iron absorption in anemia induced by, 1225–1228
Acid
aminoacetic. *See* Glycine
phosphatase, of prostatic origin in serum, *575*
titratable
in potassium depletion, *266*, *268*
in urine, and sodium loss, 1201
Acid-base balance
after carbonic anhydrase inhibitor "6063," *592*
in respiratory acidosis, 622–628
in children, 826–829, 832–833
in cyanotic congenital heart disease, 839–846
during hemodialysis, 729–735
in polycythemia vera, 52–58
effect of potassium on, *597*
effect of rubidium on, *597*
Acidosis
and effect of carbon dioxide tension on bicarbonate reabsorption, 975–977
cerebral vascular resistance in, 36–37
diabetic
alarm reaction in, *587*
erythrocyte electrolyte in, 113–119
insulin with added carbohydrate in, *599*
tissue electrolyte in, *580*
metabolic, due to renal insufficiency, 731–735
respiratory
carbonic anhydrase inhibitor in, 622–628
cellular cation transfer during, *604*
mechanical exsufflation in, 1192–1196
after ureteroenterostomy, 447
Acromegaly, and response of thyroid gland to thyroid stimulating hormone, 152, 154, 155, 156, 158
ACTH. *See* Adrenocorticotrophic hormone; Corticotropin
Addison's disease. *See* Adrenal gland, insufficiency of
Adhesion-disappearance phenomenon, *592*
Adrenal gland
carcinoma of, urinary 17-ketosteroid after cortisone in, 449–451
cortex of
and carbohydrate metabolism after stress, 781–790
and formaldehydogenic corticoid, 63, 65
steroids from, in stress, *608*
and water and electrolyte distribution, 1236–1247
cortisone acetate and desoxycorticosterone acetate in adrenalectomized rat, 1175–1179
direct estimation of steroid secretion from, 703–709
hyperplasia of, urinary 17-ketosteroid after cortisone in, 449–451
formaldehydogenic corticoid in, 63, 65
insufficiency of
electrolyte balance in, *574*
water distribution in, *574*
medullary hormones from, and cerebral blood flow, 226–232
renal excretion of corticoids from, 813–817
steroid from
intravenous, *579*
after pitressin administration, 888–889
Adrenocorticotrophic hormone
and cerebral blood flow, 372–379
continuous, for nephrotic syndrome in child, *589*
effect of, on electrolyte excretion after pitressin, 874
and leukocyte glycogen, 254, 256
mentation after, 372, 379
and plasma 17-hydroxycorticosteroids, 706–707
potassium salt in prevention of sodium retention due to, 1197–1207
psychosis after, 372, 379
variable effectiveness of, *584*
See also Corticotropin
Agglutination
differential, in study of survival of transfused erythrocytes, *576*
of tannic acid treated erythrocytes, in demonstration of type specific streptococcal antibody, 1087
Agglutination test
for Brucella agglutinin, 98–104
heterophile, *614*
Agglutinin
Brucella, testing sera for, 96–105
meningococcic, 39–43
after typhoid immunization, 693
Aging
and cerebral blood flow, 459–465
and cerebral oxygen consumption, 462–464
Air
lobar alveolar, effect of body position on concentration of, 617–621
residual, in pulmonary emphysema, 134
Albumin
in blood, 644
in copper transportation, 410–412
hyperoncotic, antidiuretic effect of, 283–291
iodinated, for measurement of plasma volume, 107–112
in lymph, 644
proteinuria after intravenous administration of, 904
radioiodine-labelled
in cirrhosis of liver, *564*
disappearance of, from tissue, 29
fate of, *554*, 746–767
in plasma, 24
in study of endothelial permeability, *601*
in serum
and calcium metabolism in osteoporosis, *574*
cationic detergents in assay of, 661–672
determination of, *600*
exchange of, with ascitic fluid, *553*
radioiodine in, 138–145
and serum cholinesterase, 468
T-1824 dye in, 138–145
turnover of, in cardiac failure, *552*
Alarm reaction, in diabetic acidosis, *587*
Alkaline phosphatase
in blood, 644
in leukocyte, 256
in lymph, 644
Alkalosis
and effect of carbon dioxide tension on bicarbonate reabsorption, 974–977
cerebral vascular resistance in, 34–37
hypokalemic, effect of potassium after nephrectomy in, 538–541
in potassium deficiency, 268
respiratory
cellular cation transfer during, *604*
during hemodialysis for uremic syndrome, 734
Allergen, and in vivo reaction with tissue protein, *564*
Aloin, 269
Alpha-ketoglutaric acid, 277
Alternating current, erythema due to irradiation produced by, 685–686
Altitude, high, blood values at, 840–842, 844
Alveolus,
lobar, effect of body position on gas concentration in, 617–621
pulmonary, in polycythemia, 57
Ambulation, and effect of oral hexamethonium on renal hemodynamics, 1134–1135, 1138
American Society for Clinical Investigation, proceedings of forty-fifth annual meeting of abstracts, 550–613
presidential address, 548–549
Amine, catechol, in postural hypotension, 1065–1069
Amino acid
in globin, 900–907
iodine-containing, in plasma, *551*
in urine, after severe burns, *592*

Aminoacetic acid. *See* Glycine
Ammonia
excretion of, after enhanced sodium reabsorption, *613*
in hepatic coma, *602*
in urine
in diabetic ketosis, 1216
after quiet standing, 245, 247
in respiratory acidosis, 623
and sodium loss, 1204
Ammonium
chloride, and cerebral circulation, 36
in solution in colon, 447
urinary excretion of, in potassium depletion due to laxatives, 266, 268
Amytal. *See* Barbiturate
Anastomosis, portacaval, and hepatic blood flow, 526–536
Anemia
aplastic, blood copper in, 335
of cancer, *560*
erythrocyte ultrastructures in, 983
filter paper electrophoresis of hemoglobin in, 1073
hemoglobin tolerance in, *603*
hemolytic,
erythropoiesis in, *567*
electrophoresis of hemoglobin in, 1073
effect of splenectomy on thalassemia major associated with, 1233–1235
in hyperthyroidism, 203
iron absorption in, 1225–1228
iron deficiency, blood copper in, 335, 336–337
microcytic hypochromic
blood copper in, 336–337
plasma iron in, 337
pernicious
blood copper in, 334–336
cerebral metabolism in, *598*
filter paper electrophoresis of hemoglobin in, 1073
primary refractory, blood copper in, 335
sickle cell, potassium in erythrocyte in, *608*
Anesthesia, and renal function, 182
Anoxia
blood values in, 837–846
in emphysema, 133–134, 135
Anti-H agglutinin, after typhoid immunization, 693
Anti-O agglutinin, after typhoid immunization, 693
Antibiotic
excretion of urinary phenols after, *598*
in intestinal degradation of choline, 1117–1120
metabolic effects of, in undernourished men, *570*
for pneumococcal pneumonia, *557*
small-colony (G) variants of Micrococcus pyogenes obtained with, *612*
staphylococci resistant to, *582*
therapeutic effect of, after cortisone, *578*
See also Aureomycin; Penicillin
Antibody
"blocking," in test for Brucella agglutinin, 100, 104
in leukemia, 317–320
after non-streptococcal antigen, as related to rheumatic fever susceptibility, 691–695
streptococcal, 630–632
type specific, 1085–1092
Antidiuresis
after hexamethonium, 176–178, 180–183
due to hyperoncotic albumin, 283–291
due to nicotine salicylate in liver disease, 422–427
due to Pitressin in liver disease, 422–427
Antidiuretic hormone
due to emotional stimulus, 289
in hepatic and cardiac disease, 931–938
after nicotine intake, 422–427
renal response in, in renal disease, *611*
in water diuresis, and solute excretion, 401–402
Antigen, non-streptococcal, rheumatic fever susceptibility as related to antibody response to, 691–695
Antigenicity, of platelets, *606*
Antiglobulin agglutination test, for Brucella agglutinin, 100–102, 104–105
Antihormone, formation of, after hog pituitary FSH, *586*
Antistreptolysin
formation of, after penicillin for streptococcal infection, 632
in immunity to streptococcal infection, *572*
Anuria, inulin space in, 209–213
Aortic valve, plastic valvular prosthesis for insufficiency of, *598*
Apnea, voluntary, and cardiac output, 6
Appetite, and dietary protein, 185–191
Apresoline
and cerebral blood flow in hypertension, 655–660
in congestive heart failure, *579*
hemodynamic alterations due to, in hypertension, *569*
intravenous, response of hypertensive kidney after, *561*
in pregnancy, 922–929
Aramine, cardiovascular effects of, *560*
Arsenate, and "L. E. cell" phenomenon, 199
Arteriole
glomerular, after protoveratrine, 87
pulmonary
in mitral valvular disease, *588*
resistance in, in emphysema, 133
Arteriosclerosis
and cerebral blood flow, 459–465
and cerebral oxygen consumption, 462–464
cerebrovascular resistance in, 700–702
and metabolism in cerebral vascular disease, *574*
Artery
pressure in
after hexamethonium, 1287, 1290
in patients with arteriovenous fistula, 543–546
See also Coronary artery; Pulmonary artery
Arthritis, rheumatoid
blood copper in, 332
composition of fibrinoid of, 1253–1259
corticosterone in, 769–773
metabolism after nitrogen mustard for, *575*
Ascitic fluid
iodo-albumin transfer in, 755–756
and plasma protein exchange, *553*
Assay, cationic detergent, of serum proteins, 661–672
Atrium
left, pulse pressure of, in mitral stenosis, *554*
right, pressure in, in arteriovenous fistula, 544–546
Aureomycin
in intestinal degradation of choline, 1117–1120
staphylococci resistant to, *582*
Auricle, right, pressure in, after hexamethonium, 1287
Autonomic nervous system, and renal function, 182
Azocoll, bacterial digestion of, 1318–1319, 1324–1326
Azotemia, in renal insufficiency, 77

B

Bacillus, in digestion of collagen, 1318–1319
Bacteria, in digestion of collagen, 1317–1322, 1323–1329
Ball valve, plastic, for severe aortic insufficiency, *598*
Ballistocardiography, vs. Fick method in determining cardiac output, 5–13, *559*
Balloon catheter, intracardiac, for producing increased venal caval pressure, 1140–1161
Barbital. *See* Barbiturate
Barbiturate
hemodialysis in poisoning due to, 364–371
urinary excretion of, 366–367
Basal metabolic rate, and cerebral oxygen consumption, 203
Base. *See* Acid-base balance
Bed rest, for infectious hepatitis, *559*
Benemid
and phenylbutazone in gout, 1128
for typhoid carriers, *593*
Beta-glucuronidase, 60
Bicarbonate
in muscle, in hypokalemic alkalotic rat, 539–541
in plasma, in hypokalemic alkalotic rat, 539–541
in potassium depletion due to laxatives, 264

Bicarbonate (*Cont'd*)
reabsorption of, and carbon dioxide tension, 972–977
in serum
in children, 825, 827
in cyanotic congenital heart disease, 839–840
in solution in colon, 446–447
in urine
in hypokalemic alkalosis, 540–541
after quiet standing, 245–249
Bile, peptidase in, 678
Bile acid, in blood, in nephrosis, 121–123
Bile duct, serum peptidase activity in disease of, 677–679
Biliary tract
disease of
and cholesterolesterifying enzyme in serum, 804, 805
serum cholinesterase in, 469
Biopsy
needle, in estimation of liver lipids, 214–224
of pleura, *597*
Blast cell, inhibition of serum desoxyribonuclease by, 196
Bleeding. *See* Hemorrhage
Blockade, autonomic ganglionic, and hexamethonium, 182
"Blocking" antibodies test, for Brucella agglutinin, 100, 104
Blood
acid-base changes in, during hemodialysis, 729–735
and adhesion-disappearance phenomenon, *592*
arterial, in polycythemia vera, 52–58
barbiturate levels in, *367*
carbon dioxide in, in pulmonary emphysema, 135
cerebral flow of, 33–37
and ACTH, 372–379
and adrenal medullary hormone, 226–232
and aging, 459–465
and arteriosclerosis, 459–465
and carbon dioxide inhalation, 696–702
and cerebral oxygen consumption, 696–702
in cerebral vascular disease, *574*
and cortisone, 372–379
in Cushing's syndrome, 375
after hydrazinophthalazine, 655–660
and hypertension, 459–465, 655–660
in hyperthyroidism, 202–207
and oxygen consumption, 696–702
in pernicious anemia, *598*
in postural hypotension, *568*
in pulmonary emphysema, 720–727
coagulation of, in multiple myeloma, *596*
constituents of, after "6063" in respiratory acidosis, 623–628
copper in, 322–327, 405–413
in disease, 329–338
in pregnancy, 329, 331
flow of
in bone marrow, 952–962
in hemorrhagic shock, *612*
after hexamethonium, 1287, 1290
after hydrazinophthalazine in hypertension, 655–660
postsympathectomy, after intravenous epinephrine, 851–856
after Valsalva maneuver, *588*
gases in
in children, 824–834
in cyanotic congenital heart disease, 837–846
glucose in, 644
after hexamethonium, 797
in rapid intravenous glucose tolerance test, 428–435
hepatic flow of
after hemorrhage, *573*
after hydrazin-ophthalazine, *586*
after norepinephrine, *605*
after portacaval shunt in cirrhosis, 526–536
intermediary metabolites in
after fructose infusion, 277–280
after glucose infusion, 277–280
leukocyte in, 642–644
metabolism in, in cerebral vascular disease, *574*
oxygen in, in children, 830–831
peripheral, 17-hydroxycorticosteroids in, 818–822
pooling of, and renal function, 1163–1169
pressure of
in adrenalectomized rat given cortisone acetate and desoxycorticosterone acetate, 1176
after Apresoline in pregnancy, 924, 926–927
arterial, after venal caval congestion, 1154, 1156, 1159
and arteriovenous fistula, 234
and dietary protein, 189, 190
and dietary sodium, 188–189
and extracellular fluid volume, 314
after hexamethonium, 173, 176–178, 181
in Laennec's cirrhosis, 1029
after potassium-deficient diet in hypertension, 633–636
See also Hypertension; Hypotension
radiosulfate disappearance from, 303–305
renal flow of
nitrous oxide vs. para-aminohippurate method in measurement of, 1180–1183
after phenylbutazone in gout, 1126, 1131
sodium in, after pitressin, 886–887, 889–891
splanchnic flow of, after hexamethonium bromide, 793–799
storage of, below 0° C. in liquid state, 1005–1011
sugar in, in diabetes mellitus, *577*
thromboplastin inhibitor in, 381–388
urea nitrogen, during globin administration, 966–967
uterine flow of, in pregnancy, *589*
venous admixture of, in mitral valvular disease, 512, 517–521
after venous congestion of limb, 1112
viscosity of, determined by Poiseuille's law
volume of
after apresoline in pregnancy, 925
body/venous hematocrit ratio in measurement of, 1309–1316
and extracellular fluid volume, 314
after intravenously administered modified human globin, 22–31
after oral hypertonic solutions following gastrectomy, *597*
radioiodine tagged globin as expander of, 22–31
See also Plasma; Serum
Blood vessel, stretching of, as stimulus for electrolyte and water excretion, 1159–1160
See also Artery; Capillary; Vein
Body fluid
alterations in, in diarrhea of infancy, 1013–1022
response of, to hypertonic saline in diabetes insipidus, *607*
See also Fluid; Water
Body weight
during administration of corticosterone in rheumatoid arthritis, 773
and dietary protein, 185–191
after globin administration, 965
in renal insufficiency, 77
Boeck's sarcoid, pulmonary function in, 909–912
Bone
calcium tolerance test in disease of, *584*
changes in, in sickle cell-hemoglobin C disease, 1279
Bone marrow, blood flow in, 952–962
Brachial artery, pressure in, in patients with arteriovenous fistula, 544, 545
Brain
blood flow in
and ACTH, 372–379
and adrenal medullary hormones, 226–232
and aging, 459–465
and arteriosclerosis, 459–465
after carbon dioxide inhalation, 696–702
and cerebral oxygen consumption, 696–702
in cerebral vascular disease, *574*
and cortisone, 372–379
in Cushing's syndrome, 375
after hydrazinophthalazine, 655–660
and hypertension, 459–465, 655–660
in hyperthyroidism, 202–207
and oxygen consumption, 696–702
in pernicious anemia, *598*

in postural hypotension, *568*
in pulmonary emphysema, 720–727
after streptokinase-streptodornase in subarachnoid space, *392*
metabolism in, in cerebral vascular disease, *574*
vascular resistance in, 33–37
Breast, ovarian function in carcinoma of, *594*
Breath, carbon-14 excretion in, 2
Breath-holding
and cardiac output, 6–7
during crying, 831
Breathing. *See* Respiration
Breathing capacity, maximal
at high altitude, 491, 494
in mitral valvular disease, 515–518, 521
Bromide space, 483–488
Bromsulfalein, 529–530
Bronchiectasis, blood copper in, *332*
Bronchus, effect of body position on lobar alveolar gas concentration in, 617–621
Brucella, testing sera for agglutinin of, 96–105
Burn, severe, urinary amino acids in, *592*

C

C^{14}. *See* Carbon, radioactive
C-antibody, in leukemia, 318
Calcium
metabolism of, during administration of corticosterone in rheumatoid arthritis, 772
in potassium depletion due to laxatives, *265*
and thromboplastin inhibitor in serum, 385–386, 387–388
tolerance test, in bone disease, *584*
in urine
during globin administration, 968
after pitressin, 875
Calcium pantothenate, for myasthenia gravis, *609*
Caloric balance and dietary protein, 188
Cancer
anemia of, *560*
prostatic, serum acid phosphatase in, 1934–1043
See also Carcinoma
Capillary
blood viscosity in, 296, 297
permeability of, in hemorrhagic shock, *612*
pulmonary, pressure in, in emphysema, 132–133
Carbohydrate
and fructose metabolism, *591*
and glucose metabolism, *591*
and insulin, in diabetic ketosis, *599*
metabolism of
and stress adrenal cortex, 781–790
and thyroid hormone, 207
and potassium metabolism, 19–21
tolerance, during administration of corticosterone in rheumatoid arthritis, 773
Carbon, radioactive, in glycine, 1–4, *554*
Carbon dioxide
arterial
at high altitude, 495
in pulmonary emphysema, 134
in blood
in blood viscosity, 296
of children, 825, 827–829
in cyanotic congenital heart disease, 833–846
during crying, 831
in emphysema, 133–135
in hyperthyroidism, 203
after *l*-nor-epinephrine, *230*
in orthostatic hypotension, *558*
in polycythemia vera, 56, 58
in pulmonary emphysema, 135
after "6063" in respiratory acidosis, 623, 627, 628
and cerebral circulation, 34–37, 696–702
cerebral
after priscoline, 127
in pulmonary emphysema, 723–727
lobar alveolar, effect of body position on concentration of, 617–621
narcosis due to, *585*
in orthostatic hypotension, *558*
in solution in colon, 446–447
tension, and bicarbonate reabsorption, 972–977
in urine, after "6063" in respiratory acidosis, 623, 627
Carbonic anhydrase
inhibitor of
acid-base equilibrium after, *592*
electrolyte metabolism after, *592*
in respiratory acidosis, 622–628
Carboxyhemoglobin, in filter paper electrophoresis of hemoglobin, 1072
Carcinoma
adrenal, urinary 17-ketosteroid after cortisone in, 449–451
of bile ducts, serum cholinesterase in, 469
of breast, ovarian function in, *594*
of head of pancreas, serum cholinesterase in, 469
of lung, cationic detergent assay of serum proteins in, 671
metastatic to liver, serum cinnamate in, 715
See also Cancer
Cardiac index, in emphysema, 133, 135
Cardiac output
after Apresoline in pregnancy, 925, 929
in cirrhosis of liver, *550*
during cold pressor test, *559*
in emphysema, 133
after hexamethonium, 1287, 1290
after leg tourniquet, 1163–1169
measurement of, by direct Fick method
vs. ballistocardiograph, 5–13
without gas analysis, 161–170
in mitral valvular disease, *588*
in patients with arteriovenous fistula, 543–546
at rest in Laennec's cirrhosis, 1025–1032
after venal caval congestion, 1160
Carditis, rheumatic, prevention of cardiac abnormalities in, *572*
Castaneda test, for Brucella agglutinin, 99–100, 104
Catechol amine, release of, in postural hypotension, 1065–1069
Catheter, intracardiac, for producing increased venal caval pressure, 1140–1161
Catheterization, cardiac, and localization of cardiac defects by dilution curves after injection of T-1824, *613*
Cation
cellular, in respiratory alkalosis, *604*
in urine, in chloride deficiency, *575*
Cell, fate of digitoxin in, *568*
Cell membrane, permeability of, to inhibitor of desoxyribonuclease, 195–196
Central nervous system, in collagen disease, *583*
Centrifugation agglutination test, for Brucella agglutinin, 101–102, 105
Cerebrospinal fluid
pressure of, and renal function, 847–849
after streptokinase-streptodornase in subarachnoid space, 391–392
Cerebrum
blood flow in, 33–37
and ACTH, *372–379*
and adrenal medullary hormones, *226–232*
and aging, 459–465
and arteriosclerosis, 459–465
after carbon dioxide inhalation, 696–702
and cerebral oxygen consumption, 696–702
in cerebral vascular disease, *574*
and cortisone, *372–379*
in Cushing's syndrome, 375
and hypertension, 459–465, 655–660
in hyperthyroidism, 202–207
after l-hydrazinophthalazine in hypertension, 655–660
and oxygen consumption, 696–702
in pernicious anemia, *598*
in postural hypotension, *568*
in pulmonary emphysema, 720–727
circulation in, and priscoline, 125–128
metabolism in, in cerebral vascular disease, *574*
vascular resistance in, 33–37
Ceruloplasmin, 405
Cetab, in assay of serum protein, 666
Cetyl dimethyl benzyl ammonium chloride, in assay of serum protein, *666*
Cetyl trimethyl ammonium chloride, in assay of serum protein, *666*
Cetylon, in assay of serum protein, 666

Child
alterations of fluid in, 1013–1022
hemoglobin in, 829
nephrotic, renal cellular electrolyte transport in, *590*
oxyhemoglobin in, with cyanotic congenital heart disease, 843
serum chloride in, 833
Chloramphenicol, *565*
Chloride
in blood, after "6063" in respiratory acidosis, 623
effect of corticotropin on, in rheumatic disease, 879–883
deficiency of, urinary cation excretion in, *575*
excretion of
and arteriovenous fistula, 236
in diabetic ketosis, 1216
and diuresis after water loading, 396–402
after hyperoncotic albumin, 283–291
after intracranial pressure-volume change, 848–849
effects of magnesium sulfate on, 858–861
after phenylbutazone in gout, 1126, 1131
after pitressin, *611*, 868–877, 934, 937
in pregnancy, 49
in renal insufficiency, 69–70
after "6063" in respiratory acidosis, 623
after vena caval congestion, 1151, 1155, 1156, 1158
after water diuresis in liver disease, 425
metabolism of, during administration of corticosterone in rheumatoid arthritis, 772
in muscle, in hypokalemic alkalotic rat, 539
in plasma, in hypokalemic alkalotic rat, 539
in potassium depletion due to laxatives, 264, 265
radioactive, and extracellular fluid volume, 483–488
radiochromic, in measurement of plasma volume, 991–995
salivary concentration gradients for, 1077–1083
in serum
of child, 833
in cyanotic congenital heart disease, 839–840
after hepatic and cardiac disease, 934, 937
in solution in colon, 444–448
space, 483–488
in measurement of extracellular fluid, 1299–1308
in sweat, 738–739, 742–744
transfer of, in adrenal insufficiency, 1238, 1241–1244
See also Electrolyte
Chloroform, inactivation of proteolytic enzyme in plasma, 478
Cholate and cholesterol, in nephrosis, 121
Choledocholithiasis, serum cholinesterase in, 469
Cholesterol
in blood, 644
and cholate, in nephrosis, 121–123
esterifying enzyme in, in liver disease, 801–806
in lymph, 644
metabolism of, in erythrocyte, 1248–1252
plasma lipid after feeding with, in rabbit treated with cortisone and hydrocortisone, *550*
Choline, antimicrobial agent in intestinal degradation of, 1117–1120
Cholinesterase, in serum
in biliary tract disease, 469
colorimetric determination of, 466–471
hepatic synthesis of, 469–470
Chondrococcus columnaris, in digestion of collagen, 1319
Chromate, sodium, in determination of red cell mass and plasma volume, 1000–1004
Chromatogram, paper, for Brucella antibody, 102–103, 105
Chromic chloride, radioactive, in determination of plasma vollume, 991–995, 1000–1004
Chromium
as erythrocyte tagging agent, *563*
radioactive
as biological tracer, *571*
in determination of erythrocyte mass, 1260–1276
in determination of erythrocyte survival, 1260–1276
in determination of plasma volume, 991–995, 1000–1004
Chromium sesquioxide, as indicator in study of intestinal absorption, *606*
Chymotrypsin, in inhibition of desoxyribonuclease, 195
Cinnamic acid, for evaluation of liver function, 711–718
Circulation. *See* Blood, flow of
Cirrhosis
biliary, serum peptidase activity in, 677–679
hepatic. *See* Liver, cirrhosis of
Cisterna magna, streptokinase-streptodornase in, 391–393
Citrate, and "L. E. cell" phenomenon, 199
Clostridium
in digestion of collagen, 1317–1319, 1324–1326
histolyticum, collagenase and proteinase from, 1324–1326
Coagulation
in activation of protein enzyme in plasma, 477–478
and fibrinolysis, *604*
intravascular, 650–653
Coccus, in digestion of collagen, 1318
Cold pressor test, cardiac output during, *559*
Collagen
bacterial digestion of, 1317–1319, 1324–1326
relation of, to fibrinoid, 1253–1259
Collagen disease
blood copper in, 336
central nervous system in, *583*
Colon, ionic composition of fluid in, 444–448
Coma, hepatic
antibiotics in, 1120
biochemical abnormalities in, *602*
Commissurotomy, mitral, pulmonary function after, 521, 1094–1096
Complement, serum, in nephrotic syndrome, *581*
Compound E, and renal tubular reabsorption, *613*
Compound F
and creatinine clearance, 815
and renal tubular reabsorption, *613*
and synovial inflammation, 480–482
Connective tissue, in maintenance of extracellular fluid composition, *583*
Consciousness, and cerebral oxygen consumption, 464
Convertin, in activation of prothrombin, 918
Coombs' test for Brucella agglutinin, 100–102, 104–105
Copper
in blood, 322–327, 405–413
in disease, 329–338
during menstruation, 324–325, 326–327
in pregnancy, 329, 331
metabolism of, 405–413
in plasma, 406–413
in disease, 329–338
in red blood cell, 408–412
Coronary artery, fibrinoid necrosis of, in streptococcus-infected rabbits, *562*
Corticoid
adrenal, and renal hemodynamics, 813–817
sodium-retaining, in human urine, *585*
urinary formaldehydogenic, 60–66
Corticotropin
effect of
on body water in rheumatic disease, 879–883
on electrolytes in rheumatic disease, 879–883
for leukemia, blood copper after, 333
potassium salt in prevention of sodium retention due to, 1197–1207
See also Adrenocorticotrophic hormone
Corticosteroid, excretion of, after pitressin administration, 887–888
Corticosterone, in rheumatoid arthritis, 769–773
Cortisone
and antibody response in leukemia, 319, 320
and cerebral blood flow, 372–379
and fluid distribution in adrenal insufficiency, 1243
glucose-insulin tolerance after, 784–790

glucose tolerance after, 783–790
insulin tolerance after, 785–790
leukocyte glycogen after, 254, 256
metabolism of in the synovial cavity, *614*
plasma lipid in cholesterol-fed rabbit treated with, *550*
potassium salt in prevention of sodium retention due to, 1197–1207
and 17-ketosteroid excretion
in Cushing's syndrome, 449–451
in urine, *578*
steroid-induced gout in rheumatoid patients after, *553*
and therapeutic effect of antibiotics, *578*

Cortisone acetate and desoxycorticosterone acetate, in adrenalectomized rat, 1175–1179

Cranium, pressure-volume changes in, and renal function, 847–849

Creatinine
clearance of
and arteriovenous fistula, 234
after compound F, 815
after hyperoncotic albumin, 286
during pitressin administration, 874
after pitressin in hepatic and cardiac disease, 932, 935
after right renal artery compression, 814–815
after water diuresis in liver disease, 426, 427
renal excretion of, and diuresis after water loading, 396–401

Creatinuria
in leukemia, *592*
in lymphoma, *592*

Cushing's syndrome
cerebral circulation in, 375
formaldehydogenic corticoid in, 63, 65
urinary 17-ketosteroids after cortisone in, 449–451

Cyanosis, due to congenital heart disease, blood gases in, 837–846

Cystine, labelled, *610*

Cytoplasmic supernatant, inhibition of desoxyribonuclease by, 194–195

D

Dead space, in mitral valvular disease, 517–518

Dehydration
and electrolyte excretion after pitressin administration, 871–874
salt solution for, in diabetic acidosis, 114

Delirium tremens, magnesium deficiency in, *568*

Dermatomyositis
blood copper in, 336
serum polysaccharide in, 1210

Desoxycorticosterone
and cortisone acetate in adrenalectomized rat, 1175–1179
and fluid distribution in adrenal insufficiency, 1243
hypertension due to tissue electrolytes in, *608*
and renal tubular reabsorption, *613*

Desoxyribonuclease
crystalline pancreatic, intravenous, *579*
human, inhibition of, 193–197, 199–200

Detergent, cationic, in fractionation of serum proteins, 661–672

Deuterium
incorporation of, in erythrocyte cholesterol, 1250
space, 1018

Dextran, and plasma volume, 340–343

Diabetes insipidus
antidiuretic effect of hyperoncotic albumin in, 289
hypertonic saline in, *607*

Diabetes mellitus
acidosis in
alarm reaction in, *587*
electrolyte in erythrocyte during, 113–119
insulin and added carbohydrate in, *599*
tissue electrolytes in, *580*
fructose in, *562*
for ketosis, 1214–1221
metabolism of, *273–280*
glucose metabolism in, *273–280*
ketone body production, after injection of sodium acetate, *584*
leukocytes in, *586*
glycogen in, *253–254*
metabolism of, 1171–1174
metabolic studies in, *577*
potassium in, 15–21
rapid intravenous glucose tolerance test in, 428–435
resistance to infection in, 441–442
serum polysaccharides in, 415–419

Dialysis, acid-base changes during, *729–735*

Diarrhea
of infancy, body fluid alterations in, 1013–1022
potassium depletion due to, 258–270

Dicumarol, in intravascular coagulation, 650–653

Diet
for infectious hepatitis, *559*
potassium-deficient, in hypertension, 633–636
protein in, and weight loss, 185–191

Digit, skin of, effect of position on oxygen tension of, 1097–1099

Digitoxin
in edema fluids, 1222–1224
intracellular fate of, *569*

Diodrast, competition of, with para-amino-hippuric acid, 1184–1190

Direct current, erythema due to irradiation produced by, 685–686

Diuresis
in hepatic and cardiac disease, 931–938
after hyperoncotic albumin, 283–291
and life situations, *602*
osmotic, 401
in hydropenia with toxemia of pregnancy, 44–50
in renal insufficiency, 76
and pitressin in hepatic and cardiac disease, 931–938
and posture, *594*
after saline ingestion, 807–811
solute, after quiet standing, 242–249
after water loading
in liver disease, 422–427
related to renal excretion of solutes, 394–402
See also Pitressin

DOCA, and renal tubular reabsorption, *613*
See also Desoxycorticosterone

Duodenum, ulcer of, electrophoretic patterns in gastric juice of, 863, 865

Dye, T-1824. *See* T-1824 dye

Dye-elution technique, in filter paper electrophoresis of hemoglobin, 1073

Dyspnea, in mitral valvular disease, 520

Dystrophy, muscular, inorganic phosphate in, 1061–1063

E

Eck fistula, episodic stupor due to, *587*

Eclampsia, renal function in, 44–50

Edema, 931–938
digitoxin in fluid of, 1222–1224
excretion of solute in, *570*
nephrotic
continuous ACTH for, *589*
renal cellular electrolyte transport in, *590*
in pregnancy, 49
pulmonary, and structure of lung membrane, 522

Electricity, energy of, in myocardium, *581*

Electrocardiography
in Laennec's cirrhosis, 1030
in myocardial disease, *595*
after oral hypertonic solutions following gastrectomy, *597*

Electroencephalogram, in pulmonary emphysema, 725

Electrolyte
effect of adrenal cortex on distribution of, 1236–1247
in blood, after "6063" in respiratory acidosis, 623, 627
effect of corticotropin on, in rheumatic disease, 879–883
distribution of, in diarrhea of infancy, 1013–1022
in erythrocyte, during diabetic acidosis, 113–119
excretion of
and arteriovenous fistula, 235–239
effects of magnesium sulfate on, 858–861
after enhanced sodium reabsorption, *613*
after oral hexamethonium, 1135–1138

Electrolyte (*Cont'd*)
in renal insufficiency, 68–78
after venous congestion of limbs, 1101–1115
effect of exogenous adrenal steroid on, after pitressin administration, 888–889
in hepatic coma, *602*
in muscle
in hypokalemic alkalotic rat, 539–541
in maintenance of extracellular fluid composition, *583*
in plasma
in hypokalemic alkalotic rat, 539–541
after oral hypertonic solutions following gastrectomy, *597*
in potassium depletion due to laxatives, *264–266*
redistribution of, in adrenal cortical insufficiency, *574*
renal cellular, in nephrotic syndrome in child, *590*
renal excretion of
and diuresis after water loading, 394–402
after splanchnicectomy, *589*
response of, to hypertonic saline in diabetes insipidus, *607*
in saliva, *569*
in serum
in cyanotic congenital heart disease, 839–840
after pitressin in hepatic and cardiac disease, 934, 937
space, 483–488
in sweat, *736–744*
in tissue, in renal hypertension, *608*
in urine
in diabetic ketosis, 1215
after hyperoncotic albumin, *283–291*
after phenylbutazone in gout, 1126, 1131
after pitressin, 868–877
after pitressin in hepatic and cardiac disease, 934, 937
after pitressin in non-edematous patients, *611*
effect of potassium on, 1197–1207
in pregnancy, 46, 49
after quiet standing, 242–249
after "6063" in respiratory acidosis, 623, 626–628
after vena caval congestion, 1145–1161
after water diuresis in liver disease, 425
and water retention, 868–877
See also Chloride; Potassium; Sodium

Electron microscope, in study of erythrocyte ultrastructure membrane, 979–990

Electrophoresis
vs. cationic detergent in assay of serum protein, 667–669
filter paper, human hemoglobin, 1070–1075
in gastric juice analysis, 862–867
in saliva analysis, 863

Emotion
and antidiuresis, 289
and renal function, 1112–1113

Emphysema
chronic pulmonary effect of mechanical exsufflation on respiratory gas exchange in, 1192–1196
pulmonary
cerebral circulation in, *720–727*
and hypertension, 132, 134, 135
at rest, 130–136
ventilation in, *570*

Empyema
blood copper in, 332

Endocarditis, bacterial, blood copper in, 332

Endothelium, permeability of, *601*

Energy, electrical, of myocardium, *581*

Enzyme
bacterial, in digestion of collagen, 1317–1319, 1324–1326
carbonic anhydrase inhibitor in respiratory acidosis, *622*
cholesterol-esterifying, in liver disease, 801–806
copper-containing, 412–413
in hydrolysis for measurement of urinary formaldehydogenic corticoid, 60–66
in inhibition of desoxyribonuclease, 195
peptidase in serum, 674–679
proteolytic
in plasma, 473–479
in serum, 477

Eosinophil, and stress, *581*

Epinephrine
action of, after apresoline in pregnancy, 926, 929
and cerebral blood flow, *226–232*
hypersensitivity to, after sympathectomy, 851–856
vs. nor-epinephrine, 231
release of, in postural hypotension, 1065–1069

Erythema, ultraviolet
of human skin, 681–689
spectrophotometric study of, 1053–1059

Erythrocyte
and adhesion-disappearance phenomenon, *592*
and blood viscosity, 297
cholesterol metabolism in, 1248–1252
chromium as tagging agent for, *563*
coated with nonspecies specific bacterial products, serum hemolysin for, *596*
copper in, *322–327*, 408–412
in infection, 329–337
electrolyte in, during diabetic acidosis, 113–119
electron microscopic study of ultrastructures of, 979–990
in hypokaliemia due to laxatives, *266*
irradiated, 987

Erythrocyte
labeled
with heavy nitrogen, *578*
modified, 983
phosphorus in, 499–500, 503, 508, 509
for measurement of plasma volume, 111
with radiochromate, *571*
with radiophosphorus, *26*
life span of, 1250
in lymph, 641, 648
mass of, radiochromium in determination of, 1000–1004
potassium exchange in, in sickle cell anemia, *608*
sedimentation rates, in rheumatoid arthritis and degenerative joint disease, 1212
sensitized, 987
stored, 987
survival of
measured by chromium tagging, 1260–1276
measured by selective agglutination, 1265–1266
after transfusion, *576*
tannic acid treated, in demonstration of type specific antibody, 1085–1092
transfused
compatible, destruction of, *607*
in thalassemia major, 1229–1233
trypinized, 985
volume of, body/venous hematocrit ratio in measurement of, 1309–1317
water in, in diabetic acidosis, 118–119

Erythromycin, for pneumococcal pneumonia, *557*

Erythropoiesis, in hemolytic anemia, *567*

Escherichia coli, respiration of, after streptomycin, *593*

Ester, cholesterol, in liver disease, 801–806

Estrogen
and protein bound iodine of serum, *565*
therapy with, and serum acid phosphatase in prostatic cancer, 1039

Euthyroidism, radioiodine metabolism in, after thyroid stimulating hormone, 149–159

Evans blue dye. *See* T-1824 dye

Exercise
arterial saturation after, 831
in congestive heart failure, *579*
effects of, on cerebral metabolic functions, *724*
and respiratory gas values, at high altitude, 491, 495

Exercise minute ventilation and gas exchange, at high altitude, 491, 495

Exercise tolerance test, 495

Exsufflation, and respiratory gas exchange, 1192–1196

Extracellular fluid
and diuresis, 401–402
maintenance of composition of, *583*

F

Fasting, and leukocyte glycogen, 253

Fatty acid, in liver, and hepatic lipids, 221

Feces, carbon-14 excretion in, 2

Fibrin
as substrate for proteolytic enzyme of plasma, 473–479
width of strand of, and platelet concentration, *571*
Fibrinogen, as substrate for proteolytic enzyme of plasma, 473–479
Fibrinoid, composition of, in rheumatoid arthritis, 1253–1259
Fibrinolysin, substrates for, 473–479
Fibrinolysis, and coagulation, *604*
Fibrosis, pulmonary, cerebral metabolism in, 725
Fick method
vs. ballistocardiograph, in measurement of cardiac output, 5–13, *559*
direct, without gas analysis, in determination of cardiac output, 161–170
direct vs. indirect, in renal blood flow measurement, 1180–1183
Filter paper electrophoresis, of hemoglobin, 1070–1075
Filtration, glomerular
and arteriovenous fistula, 237–238
after hexamethonium, 174–183, 1293
after leg tourniquet, 1167, 1169
after phenylbutazone in gout, 1126, 1131
after protoveratrine, 81, 86
after quiet standing, 245
after sodium deprivation in renal insufficiency, 72–74, 76
after vena caval congestion, 1151, 1155, 1156
after venous congestion of limbs, 1114
Fistula
arteriovenous
and blood pressure, 234
cardiodynamics of, 543–546
and renal hemodynamics, 233–239
Eck, episodic stupor due to, *587*
Fluid
ascitic
iodo-albumin transfer in, 755–756
and plasma protein exchange, *553*
change in ionic composition of, in colon, 444–448
of edema, digitoxin in, 1222–1224
extracellular
and diuresis, 401–402
in hypertension, 312–316
maintenance of composition of, *583*
measurement of, 1299–1308
radiosulfate in measurement of, 299–309, 312–316
peritoneal, agglutination of granulocytes in, 437
synovial, after intra-articular hydrocortisone, 480–482
See also Water
Folin method, in filter paper electrophoresis of hemoglobin, 1072–1073
Follicle stimulating hormone, from hog pituitary, in antihormone formation, *586*
Fractionation, cationic detergent, of serum proteins, 661–672
Fructose
for diabetic ketosis, *562*, 1214–1221
diabetic leukocytes after, *586*
in Embden-Meyerhof scheme, 279–286
and leukocyte metabolism, 1171–1174
metabolism of
after carbohydrate deprivation, *591*
in hepatic disease and diabetes mellitus, 273–280
FSH, from hog pituitary, in antihormone formation, *586*

G

Gamma globulin. *See* Globulin
Ganglion, autonomic, hexamethonium-induced blockade of, 182
Gantrisin, in intestinal degradation of choline, 1117–1118
Gas
in blood, in children, 824–834
lobar alveolar, effect of body position on concentration of, 617–621
Gas exchange
and minute ventilation, at high altitude, 491–495
respiratory, effect of mechanical exsufflation on, 1192–1196
Gastrectomy, oral hypertonic solutions after, *597*
Gastric juice, electrophoretic analysis of, 862–867
Gastrointestinal tract
inulin in, 212
neoplasia of, *600*
upper, effect of gastric acidity on absorption of iron from, *571*
Globin
and liver, 30
modified human, nutritive adequacy of, 899–907
as protein supplement, 964–971
Globulin
β_1 metal-binding, in copper transportation, 410–412
in blood, 644
gamma
half-life of, in hepatic cirrhosis, *573*
radioiodine labeled, in cirrhosis of liver, *564*
turnover of, in cardiac failure, *552*
in lymph, 644
in serum, cationic detergents in assay of, 661–672
Glomerular filtration rate. *See* Filtration
Glomerulonephritis
acute
and serological type of streptococcus, 348–355, 359–363
after streptococcal infections, *606*
variations in incidence of, 345–355
vs. rheumatic fever, 362
Glomerulus
arteriolar resistance of, after protoveratrine, 87
filtration of. *See* Filtration
Glucosamine, in serum, 416
Glucose
in blood, 644
cerebral
and ACTH, 373, 377
and cortisone, 373, 377
and priscoline, 127
diabetic leukocytes after, *586*
vs. fructose, in diabetic ketosis, 1217
hepatic output of, after hexamethonium, 797
hypertonic, after gastrectomy, *597*
intravenous, inorganic phosphate response to, in muscular dystrophy, 1061–1063
and leukocyte metabolism, 1171–1174
in lymph, 644
metabolism of
after carbohydrate deprivation, *591*
in hepatic disease and diabetes mellitus, 273–280
muscle uptake of, after insulin, *593*
and plasma radiophosphate disappearance, 500
and serum inulinoid blank, 212
tolerance
during administration of corticosterone in rheumatoid arthritis, 773
after cortisone, 783–790
in fatty liver, *565*
rapid intravenous, 428–435
Glucose-insulin tolerance test, after cortisone, 784–790
Glucuronic acid
in inhibition of β-glucuronidase, 60
after intravenous cinnamate, 716, 718
Glucuronidase, beta, 60
Glucuronide, urinary corticoid, hydrolysis of, 60–66
Glutamine, in hepatic coma, *602*
Glycine
labeled with carbon-14 methylene, 1–4
labeled with radio-carbon, *554*
nitrogen, for uric acid synthesis, 775–777
Glycogen
in attraction of granulocyte, 441
deposition of, in fatty liver, *565*
in human leukocytes, 251–256
Glycogenesis, and potassium, 20
Glycogenolysis, and potassium, 20
Glycoside. *See* Digitoxin
Glycylglycine, serum hydrolysis of, 674–679
Glycylglycylglycine, serum hydrolysis of, 674–679
Glycyl-L-tyrosine, serum hydrolysis of, 674–679
Gout
glycine nitrogen for uric acid synthesis in, 775–777
nitrogen for uric acid synthesis in, 778–780

Gout (*Cont'd*)
effect of phenylbutazone on renal clearance of urate in, 1121–1132
renal clearance in, after phenylbutazone, *605*
serum polysaccharide in, 1210
steroid induced, in rheumatoid patients after cortisone therapy, *553*
Granulocyte
chemotactic behavior of, 436–442
inhibition of serum desoxyribonuclease by, 196, 200
Graves' disease, thyrotrophin in, 151
Group A streptococcus, serological type of
and acute glomerulonephritis, 348–355, 359–363
and rheumatic fever, 354–355

H

H-agglutinin, after typhoid immunization, 693
Hatschek's formula, in determination of blood viscosity, 293, 296
Heart
abnormalities of, after rheumatic carditis, *572*
effect of aramine on, *560*
congestive failure of, *579*
antidiuretic hormone in, 931–938
cyanotic congenital disease of, blood gases in, 837–846
after hexamethonium, *572*
intracardiac catheter, for raising increased vena caval pressure, 1140–1161
intracellular fate of digitoxin in, *569*
metabolism of, *556*
output of. *See* Cardiac output
pressure in, and arteriovenous fistula, 543–546
rate of
and arteriovenous fistula, 234
after hexamethonium, 1287
in Laennec's cirrhosis, 1029
rhythm of, controlled by external electric pacemaker, *614*
size of, in Laennec's cirrhosis, 1029
Heart disease
congenital, polycythemia of, 56, 57
sodium retention in, *566*
water retention in, *566*
Heart failure
albumin turnover in, *552*
gamma globulin turnover in, *552*
Hemagglutination, with tannic acid, in demonstration of type specific streptococcal antibody, 1085–1092
Hematocrit
and blood viscosity, 293, 296–297
body/venous ratio, 1309–1316
after globin administration, 965
Hemochromatosis, blood copper in, 334
Hemodialysis
acid-base changes during, 729–735
in barbiturate poisoning, 364–371
Hemodilution, and tonicity of intravenous saline, 34
Hemoglobin
C, 1277–1284
in children, 829
concentration of, in hyperthyroidism, 203, 205, 206
filter paper electrophoresis of, 1070–1075
infused, in anemia, *603*
in polycythemia vera, 55, 57
radiocarbon in, *554*
sickle cell, 1277–1284
Hemolysin, in serum, for erythrocytes coated with nonspecies specific bacterial products, *596*
Hemolysis
differential, in study of survival of transfused erythrocytes, *576*
inhibition of, in study of hyperlipemia, *607*
in primaquine-sensitive Negro, *609*
of stored blood, 1007–1011
Hemolytic anemia, *567*, 1073, 1229–1233
Hemolytic disease, hereditary nonspherocytic, *591*
Hemolytic test, for tuberculosis, *590*
Hemorrhage
due to deficiency of plasma thromboplastin factors, *599*
dextran after, 341, 342
dual hemostatic defect in pseudohemophilia, *551*
and hepatic blood flow, *573*
shock due to, blood flow and transcapillary exchange in, *612*
and splanchnic oxygen consumption, *573*
Hemostasis, dual defect of, in pseudohemophilia, *551*
Heparin
in intravascular coagulation, 650–653
and serum lipids, *566*
Heparinoid, synthetic, and serum lipids, *566*
Hepatitis
acute, plasma lipoprotein in, *564*
acute infectious, serum peptidase activity in, 677–679
acute viral, colorimetric determination of serum cholinesterase in, 466–471
and cholesterol-esterifying enzyme in serum, 801–803, 805–806
fructose metabolism in, 273–280
glucose metabolism in, 273–280
infectious
bed rest for, *559*
diet for, *559*
serum cinnamate in, 717
after use of pooled plasma, *601*
Hepatolenticular degeneration, blood copper in, 336
Heredity
of hemoglobin C, 1277–1284
hypercholesterolemia, *611*
Heterophile agglutination test, *614*
Hexamethonium
hemodynamic alterations due to, in hypertension, *569*
hemodynamic effects of, 1285–1298
in hypertension, *572*, 1133–1139
Hexamethonium bromide, and splanchnic blood flow, 793–799, 1292
Hexamethonium chloride, and renal function, 172–183
Hexose
and leukocyte metabolism, 1172–1173
in serum, in diabetic ketosis, 1215
High altitude, blood values at, 840–842, 844
Hippuric acid
conversion of cinnamic acid to, 714
synthesis of, *554*
Histamine, epinephrine excretion after, in postural hypotension, 1068
Hodgkin's disease
antibody response in, 319
blood copper in, 334
blood flow in marrow in, 957
and cholesterol-esterifying enzyme in serum, 804
Hormone
adrenal cortical, direct estimation of, 703–709
adrenal medullary, and cerebral blood flow, 226–232
adrenocorticotrophic. *See* Adrenocorticotrophic hormone; Corticotropin
antidiuretic
due to emotional stimulus, 289
in hepatic and cardiac disease, 931–938
after nicotine intake, 422–427
in water diuresis and solute excretion, 401–402
follicle-stimulating, in antihormone formation, *586*
thyroid, 149–159
exchangeable pool of, 453–457
synthesis of, *577*
utilization of, *558*
See also Thyroxine
thyroid stimulating, and radioiodine metabolism, 149–159
Huddleson test, for Brucella agglutinin, 98–99, 104
Hyaluronic acid, in joint fluid, after hydrocortisone, 480–482
Hydration, and electrolyte excretion after pitressin administration, 871–874
Hydrazinophthalazine
and cerebral blood flow in hypertension, 655–660
chemical observations on, *595*
hemodynamic alterations due to, in hypertension, *569*
and hepatic blood flow in dog, *586*
l-, in hypertension, *572*
in pregnancy, 922–929
Hydrocortisone
and fluid distribution in adrenal insufficiency, 1243
metabolism of, in synovial cavity, *614*
plasma lipid in cholesterol-fed rabbit treated with, *550*
and synovial inflammation, 480–482
Hydrogen ion, in hypokalemic alkalosis, 540–541

Hydrolysis, enzymatic, for measurement of urinary formaldehydogenic corticoids, 60–66
Hydropenia, osmotic diuresis in, with toxemia of pregnancy, 44–50
Hydroxycorticosteroid
in blood, 818–822
in stress, *608*
Hydroxyproline, in fibrinoid, 1254, 1257
Hypercapnia, in emphysema, 133–135
Hypercholatemia, 121–123
Hypercholesterolemia
heredity of, *611*
in serum cholate, 121
Hypercorticoidism, 65
Hypercorticoiduria, 65
Hypercupremia, in infection, 332–333
Hyperlipemia, hemolysis inhibition in study of, *607*
Hypertension
arterial, hepatocellular dysfunction in, *561*
blood pressure in, after hexamethonium, 173, 176–178, 181
and cerebral blood flow, 459–465
metabolism in cerebral vascular disease in, *574*
after hydrazinophthalazine, 655–660
and cerebral oxygen consumption, 462–464
cerebrovascular resistance in, 700–702
DCA, tissue electrolytes in, *608*
essential, *556*
apresoline in pregnancy with, 924
carbon dioxide as cerebral vasodilator in, 701
extracellular fluid volume in, 312–316
formaldehydogenic corticoid in, 63, 65
glomerular filtration rate in, after hexamethonium, 176
hexamethonium in, *572*
hydrazinophthalazine in, *572*
muscle blood flow in, after hexamethonium, 1290–1291
and norepinephrine, 226, 230
potassium-deficient diet in, 633–636
protein diet in, 185–191
pulmonary, 130–136
in emphysema, 132, 134, 135
renal, tissue electrolytes in, *608*
renal function in, after hexamethonium, 1133–1139
and effect of venous congestion of limbs upon renal function, 1110, 1112, 1113, 1114
Hyperthyroidism
blood copper in, 335
cerebral blood flow in, 202–207
oxygen consumption in, 202–207
radioiodine metabolism in, after thyroid stimulating hormone, 149–159
Hyperventilation
during crying, 831
in cyanotic congenital heart disease, 844
after norepinephrine, 230
in orthostatic hypotension, *558*
after priscoline, 127
Hypocapnia, in orthostatic hypotension, *558*
Hypoglycemia, due to insulin in postural hypotension, 1068, 1069
Hypokaliemia, due to laxatives, 258–270
Hypotension
due to hexamethonium, 1285–1298
orthostatic, hyperventilation-induced hypocapnia in, *558*
postural
cerebral hemodynamics in, *568*
epinephrine in, 1065–1069
nor-epinephrine in, 1065–1069
protoveratrine-induced, and renal function, 80–88
Hypothyroidism, blood copper in, 336

I

Index, cardiac, in emphysema, 133, 135
Infant. *See* Child
Infection
blood copper in, 329–333
neutrophilic leukocytosis of, 255
streptococcal, immune response in, 630–632
Inflammation
serum complement as indication of, *568*
synovial, hydrocortisone in, 480
Influenza, antibody response of rheumatics to immunization against, 692
Insufficiency, of mitral valve vs. stenosis, *595*
Insulin
and added carbohydrate in diabetic ketosis, *599*
diabetic leukocytes after, *586*
epinephrine excretion after, in postural hypotension, 1067–1069
exogenous, and urinary excretion of sugar and nitrogen in diabetes mellitus, *577*
and fructose
for diabetic ketosis, 1214–1221
metabolism of, 276, 279
and glucose 276, 279
muscle uptake, *593*
and leukocyte metabolism, 1171–1174
nor-epinephrine excretion after, in postural hypotension, 1067–1069
and plasma radiophosphate disappearance, 500
Insulin tolerance test, after cortisone, 784–790
Intestinal tract
antimicrobial agents in degradation of choline in, 1117–1120
indicator method for study of absorption from, *606*
Isoantibody, in destruction of transfused incompatible erythrocytes, *607*
Inulin, 75–76
clearance of, 90–94
and arteriovenous fistula, 234
during pitressin administration, 874
after sodium deprivation in renal insufficiency, 72–73
after venous congestion of limbs, 1114
plasma concentration of, 90–94
space, 1013–1022
in adrenal insufficiency, 1237, 1238–1244
in anurea, 209–213
compared with simultaneous radiosulfate space, 307
in measurement of extracellular fluid, 1299–1308
Iodide, salivary concentration gradients for, 1077–1083
Iodine
number, of fatty acids in liver, 221
protein-bound
estrogen influence on, *565*
in thyroid disease, 149–159
radioactive
albumin tagged with, 29, *601*, 746–767
in blood flow in bone marrow, 952–962
globin tagged with, 22–31
in human serum albumin, 138–145
for measurement of plasma volume, 107–112
salivary concentration gradients for, 1077–1083
and thyroid stimulating hormone, 149–159
in study of thyroidal function, *577*
thyroxine labeled with, *596*
triiodothyronine labeled with, *596*
urinary excretion of, in thyroid disease, 153
in serum, *565*
and utilization of thyroid hormone, *558*
Iron
absorption of, in anemia, 1225–1228
effect of gastric acidity on absorption of, *571*
in plasma, in disease, 331–337
Iron deficiency anemia, filter paper electrophoresis of hemoglobin in, 1073
Irradiation, ultraviolet, ythematous response of human skin to, 681–689
Isoleucine, in globin, 900–901
Isosthenuria, in potassium depletion, 269

J

Jaundice
colorimetric determination of serum cholinesterase in, 468–471
due to obstructing neoplasm, and cholesterol-esterifying enzyme in serum, 804, 805
obstructive, serum peptidase activity in, 677–679

Joint
hydrocortisone for synovial inflammation, 480
metabolism of cortisone in, *614*
serum polysaccharide in degenerative disease of, 1208–1213

K

Keto glutaric acid, alpha
after fructose infusion, 277–278, 279–280
after glucose infusion, 277–278, 279–280
Ketone
production of, after injection of sodium acetate, *584*
splanchnic, and intravenous sodium octanoate, *610*
Ketosis, diabetic
fructose for, *562*, 1214–1221
insulin with added carbohydrate in, *599*
Ketosteroid
alpha, excretion pattern of, 940–948
excretion of, during administration of corticosterone in rheumatoid arthritis, 773
in urine
after cortisone, *578*
after cortisone in Cushing's syndrome, 449–451
Kidney
adjustment of, to acidifying salt after sodium depletion, *602*
artificial
acid-base changes during use of, 729–735
in barbiturate poisoning, 364–371
bicarbonate reabsorption by, and carbon dioxide tension, 972–977
blood flow through
after hexamethonium, 1289, 1293
nitrous oxide vs. para-amino-hippurate method in measurement of, 1180–1183
after phenylbutazone in gout, 1126, 1131
cellular electrolyte transport systems in, *590*
diodrast excretion by, 1184
electrolyte excretion by
after splanchnicectomy, *589*
after vena caval congestion, 1145–1161
function of
after acute renal failure, *567*
in diarrhea of infancy, 1013–1022
after hexamethonium in hypertension, 1133–1139, 1293
effect of intracranial pressure-volume change on, 847–849
and leg tourniquets, 1163–1169
and life situations, *602*
effects of magnesium sulfate on, 858–861
after phenylbutazone in gout, *605*
during pitressin administration, 874
effect of position on, *582*
in potassium depletion due to laxatives, 268–269
in potassium repletion for hypokaliemia, 266–267, 269
and venous congestion of limbs, 1101–1115
hemodynamics of
and renal excretion of adrenal corticoids, 813–817
after arteriovenous fistula, 233–239
and hexamethonium, 172–183
after hydrazinophthalazine, *572*
hypertensive, after intravenous hydrazinophthalazine, *561*
hypokalemic alkalosis in nephrectomized rats, 538–541
insufficiency of
and chloride excretion, 69–70
and electrolyte excretion, 69–72, 76–77
and potassium excretion, 69–71
and sodium deprivation, 68–78
and water excretion, 69, 76–77
para-amino-hippurate excretion by, 1184
effect of phenylbutazone on urate clearance by, 1121–1132
plasma flow in
after hexamethonium, 1293
after protoveratrine, 81–86, 88
after salt deprivation, 76
in pregnancy, 44–50
and protoveratrine, 80–88
response of
to antidiuretic hormone, in renal disease, *611*
to hypertonic saline in diabetes insipidus, *607*
solute excretion by
and diuresis after water loading, 394–402
after quiet standing, 242–249
water excretion by, after vena caval congestion, 1145–1161
Korotkoff sounds, *598*

L

Lactic acid
arterial concentration of, after hexamethonium, *797*
after fructose infusion, 277–278, 279–280
after glucose infusion, 277–278, 279–280
in serum, in cyanotic congenital heart disease, 839
Laennec's cirrhosis, cardiac output at rest in, 1025–1032
Lanatoside C, hemodynamic effects of, *563*
Laxative, chronic potassium depletion due to, 258–270
L. E. cell
production of, in normal individuals, *558*
varying susceptibility of leukocyte to, *559*
Leucylglycine, serum hydrolysis of, 674–679
Leukemia
anomalous serum protein in, *600*
antibody response in, 317–320
blastic, 255
blood copper in, 333–334
blood flow in marrow in, 957–962
chronic lymphocytic, leukocyte glycogen in, 255, 256
chronic myelocytic, leukocyte glycogen in, 255, 256
creatinuria in, *592*
erythrocyte ultrastructures in, 983
leukocyte in, utilization of radioactive cystine by, *610*
leukocyte glycogen in, 255–256
leukocyte phosphorus turnover in, *553*
6-mercaptopurine for, *557*
thoracic duct lymph in, 638–649
Leukocyte
abnormal, immunologic mechanisms of, *567*
alkaline phosphatase in, 256
in blood, 642–644
in diabetes mellitus, *586*
glycogen in, 251–256
leukemic
phosphorus turnover in, *553*
utilization of radioactive cystine by, *610*
metabolism of, 1171–1174
effect of saccharides on movement of, 436–442
in spinal fluid after streptokinase-streptodornase solutions in subarachnoid space, 391–392
susceptibility of, to "L.E. cell" transformation, 197
in thoracic duct lymph, 641–644
varying susceptibility of, to L. E. phenomenon, *559*
See also White blood cell
Leukocytosis, neutrophilic, leukocyte glycogen in, 255
Light, ultraviolet, erythematous response of human skin to, 681–689
Limb, venous congestion of, and renal function, 1101–1115
Lipid
in liver, needle biopsy in estimation of, 214–224
in plasma
in acute hepatitis, *564*
in cholesterol-fed rabbit treated with cortisone and hydrocortisone, *550*
in serum
effect of heparin in, *566*
effect of synthetic heparinoid on, *566*
Lipoprotein, in plasma, in acute hepatitis, *564*
Liver
blood flow in
after hemorrhage, *573*
after hexamethonium, 1289, 1292
after hydrazinophthalazine, *586*
after norepinephrine, *605*
after portacaval shunt in cirrhosis, 526–536
cirrhosis of
antidiuretic hormone in, 931–938
cardiac output in, *550*
cardiac output at rest in, 1025–1032
and cholesterol-esterifying enzyme in serum, 803, 805
colorimetric determination of serum cholinesterase in, 466–471

fructose metabolism in, 273–280
glucose metabolism in, 273–280
half life of gamma globulin in, *573*
and hepatic blood flow after portacaval shunt, 526–536
Laennec's, electrocardiography in, 1030
pitressin tolerance in, 885–891
radioiodine-labelled albumin in, *564*
radioiodine-labelled gamma globulin in, *564*
serum cinnamate in, 715, 717
serum peptidase activity in, 677–679
sodium retention in, *560*
colorimetric determination of serum cholinesterase in disease of, 466–471
coma
antibiotics in, 1120
biochemical abnormalities in, *602*
constituents of, in hepatic disease, *603*
disease of
and cholesterol-esterifying enzyme in serum, 801–806
serum peptidase activity in, 677–679
and water diuresis, 422–427
fatty
glucose tolerance in, *565*
glycogen deposition in, *565*
function of, cinnamic acid for evaluation of, 711–718
and globin, 30
glucose output of, after hexamethonium, 797
hepatocellular dysfunction in arterial hypertension, *561*
in inactivation of antidiuretic hormone, *590*
intracellular fate of digitoxin in, *569*
lipids in, needle biopsy in estimation of, 214–224
mitochondria of, potassium metabolism by, *591*
oxygen uptake by, following portacaval anastomosis, 530–531
parenchymal disease of
fructose metabolism in, 273–280
glucose metabolism in, 273–280
sarcoidosis of, cationic detergent assay of serum protein in, 664

Lobe, effect of body position on alveolar gas concentration in, 617–621

Lung
arteriolar resistance in, in emphysema, 133
effect of body position on lobar alveolar gas concentration in, 617–621
carcinoma of, cationic detergent assay of serum proteins in, 671
elasticity of, *588*
emphysema of, 130–136
cerebral circulation in, 720–727
effect of mechanical exsufflation on respiratory gas exchange in, 1192–1196
in excretion of carbon-14, 2
fibrosis, cerebral metabolism in, 725
function of
in Boeck's sarcoid, 909–912
before and after commissurotomy for mitral stenosis, 1094–1096
spirographic evaluation of, prior to surgery, *571*
in mitral valvular disease, 510–524
physiology of, at high altitudes, 490–496
in polycythemia, 57
resistance of, *588*
ventilation of, in emphysema, *570*

Lupus erythematosus, disseminated, serum polysaccharides in, 1210

Lupus erythematosus cell phenomenon, inhibition of, 197–201

Lymph, from thoracic duct, 637–649

Lymphoblast, 647

Lymphoblastoma, blood flow in marrow in, 957, 958

Lymphocyte, 642–649
inhibition of serum desoxyribonuclease by, 196, 200
life span of, 644, 647

Lymphoma
creatinuria in, *592*
malignant, and anomalous serum protein, *600*

Lymphomata, malignant, antibody response in, 317–320

Lymphosarcoma
antibody response in, 319
blood copper in, 334

M

M protein, of group A streptococci, type-specific antibodies against, 1085–1092

Magnamycin, for pneumococcal pneumonia, *557*

Magnesium
clearance of, and electrolyte excretion, 858–861
deficiency of, in delirium tremens, *568*
and "L. E. cell" phenomenon, 199

Mannitol
clearance of, in pregnancy, 47
in measurement of extracellular fluid volume, 312–316
and solute diuresis during quiet standing, 245–249

Marrow, blood flow in, 952–962

Maximal breathing capacity
at high altitude, 491, 494
in mitral valvular disease, 515–518, 521

Melanin, reflection spectrum of, after irradiation, 1055–1059

Meningococcus, agglutinin for, 39–43

Menstruation, blood copper during, 324–325, 326–327

Mentation, after ACTH, 372, 379

Mercaptopurine, for leukemia, *557*

Mesobilirubinogen, *585*

Metabolite, intermediary
after fructose infusion, 277–280
after glucose infusion, 277–280

Metastasis, to liver, serum cinnamate in, 715

Methemoglobin, in polycythemia, 56

Methylene, labeled with radioactive carbon, 1–4

Micrococcus pyogenes, small-colony (G) variants of, obtained with antibiotics, *612*

Minute ventilation, and gas exchange, at high altitude, 491–495

Mitochondria, of liver, potassium metabolism by, *591*

Mitral valve
insufficiency of
and cardiac output, *588*
vs. stenosis, *595*
pulmonary function in disease of, 510–524
stenosis of
cardiac output in, *588*
vs. insufficiency, *595*
pulmonary arteriolar resistance in, *588*
pulmonary function in, 510–524
pulmonary function in, after commissurotomy for, 1094–1096
pressure pulse wave in, *554*
pulmonary pressure in, 134–135
of rheumatic origin, *566*

Monocyte, inhibition of serum desoxyribonuclease by, 196

Mononucleosis, infectious, leukocyte glycogen in, 255

Mucoprotein
glandular, electrophoretic pattern of, 864, 866
in rheumatoid arthritis vs. degenerative joint disease, 1208–1213
in serum, in diabetes mellitus, 417, 419

Mucoproteose, electrophoretic pattern of, 864, 867

Muscle
blood flow through, after hexamethonium, 1286, 1290
electrolytes in
in hypokalemic alkalotic rat, 539
in maintenance of extracellular fluid composition, *583*
glucose uptake by, after insulin, *593*
inorganic phosphate in dystrophy of, 1061–1963
measurement of extracellular phase of, 1299–1308

Mustard, nitrogen, for rheumatoid arthritis, *575*

Myasthenia gravis, calcium pantothenate in, *609*

Myeloid cell, glycogen in, 251–256

Myeloma, multiple
blood flow in marrow in, 957, 958, 961
delayed blood coagulation in, *596*
serum cinnamate in, 716, 718

Myelomatosis, cationic detergent assay of serum proteins in, 671

Myeloproliferative syndrome, leukocyte glycogen in, 255

Myocardium
electrical energetics of, *581*
electrocardiography in disease of, *595*
Myxedema
l-thyroxine in, *552*
l-triiodothyronine in, *552*
Myxobacteria, in digestion of collagen, 1319

N

Narcosis, due to carbon dioxide, *585*
Neck, compression of, and renal function, 847–849
Needle biopsy, in estimation of liver lipids, 214–224
Negro
hemoglobin C in, 1277–1284
primaquine-sensitive hemolysis in, *609*
Nembutal. *See* Barbiturate
Neoplasia, gastrointestinal, *600*
Neoplasm, obstructive jaundice due to, and cholesterol-esterifying enzyme in serum, 804, 805
Nephrectomy, inulin space after, 211–213
Nephritis
epidemic, 345–355
after scarlet fever, 345–348
and serological type of streptococcus, 348–355
Nephron
in renal disease, 181
in renal insufficiency, 77
Nephrosis
cholate cholesterol relationship in, 121–123
"lipoid," blood copper in, 336
Nephrotic syndrome
in child
continuous ACTH for, *589*
renal cellular electrolyte transport in, *590*
serum complement in, *581*
Nervous system
autonomic, and renal function, 182
central, in collagen disease, *583*
sympathetic, and renal function, 182
Newborn. *See* Child
Nicotine salicylate, antidiuresis due to, in liver disease, 422–427
Nitrogen
blood urea, during globin administration, 966–967
glycine, for uric acid synthesis in, 775–777
heavy, posttransfusion metabolism of erythrocytes labelled with, *578*
metabolism of
during corticosterone administration in rheumatoid arthritis, 772
during globin administration, 900–907, 967
in potassium depletion due to laxatives, 265
retention of, after sodium deprivation in renal insufficiency, 73
in sweat, 740–744
for uric acid synthesis in gout, 778–780
in urine in diabetes mellitus, *577*
Nitrogen mustard, for rheumatoid arthritis, *575*
Nitrous oxide method
in measurement of cerebral blood flow, 463–464
vs. para-amino-hippurate method, in renal blood flow measurement, 1180–1183
Norepinephrine
and cerebral blood flow, 226–232
vs. epinephrine, 231
and hepatic blood flow, *605*
after hexamethonium, 178–180
release of, in postural hypotension, 1065–1069

O

O-agglutinin, after typhoid immunization, 693
Octab, in assay of serum proteins, 661–672
Octadecyl dimethyl benzyl ammonium chloride, in assay of serum proteins, 661–672
Octadecyl dimethyl ethyl ammonium chloride, in assay of serum protein, *666*
Octimet, in assay of serum protein, *666*
Oliguria
inulin space in, 209–213
after protoveratrine, *87*
Oncotic pressure, of plasma, as antidiuresis stimulus, 283–291
Osmolarity, and erythrocyte water, 118
Osteoporosis, calcium metabolism in, *574*
Output, cardiac. *See* Cardiac output
Ovary, and breast carcinoma. *594*
Oxalate
and "L. E. cell" phenomenon, 199
and thromboplastin inhibitor in serum, 386
Oxygen
administered, in chronic pulmonary emphysema, 1193
alveolar, at high altitude, 495
arterial
at high altitude, 495
in polycythemia vera, 52–58
arterial-hepatic venous difference, after hexamethonium, 796, 799
in blood, of children, 825, 830–831
in cyanotic heart disease, 837–846
after norepinephrine, 230
cerebral
and ACTH, 373, 377
and cortisone, 373, 377
cerebral
after hydrazinophthalazine in hypertension, 655–660
after priscoline, 127
cerebral consumption of, 720–727
and aging, 462–464
and arteriosclerosis, 462–465
and cerebral blood flow, 696–702
and hypertension, 462–464
consumption of, in hyperthyroidism, 202–207
hepatic uptake of, following portacaval anastomosis, 530–531
lobar alveolar, effect of body position on concentration of, 617–621
in pulmonary function after commissurotomy for mitral stenosis, 1094–1096
splanchnic consumption of
after hexamethonium, 796, 799
during hypotension due to bleeding, *573*
tension of, in digital skin, 1097–1099
Oxyhemoglobin
in blood, in cyanotic congenital heart disease, 837–846
in children, 825
Oxyhemoglobin dissociation curve of blood, in polycythemia vera, 55, 57

P

Pacemaker, external electric, in control of cardiac rhythm, *614*
Pain, due to hypertonic saline in supportive tissues, 1045–1051
Pancreas
desoxyribonuclease from, *579*
serum cholinesterase in carcinoma of head of, 469
Papain, in inhibition of desoxyribonuclease, 195
Paper chromatogram, for Brucella agglutinin, 102–103, 105
Para-acetylaminohippuric acid in measurement of cardiac output by direct Fick principle without gas analysis, 162–170
Para-aminohippurate
clearance of, during pitressin administration, 874
after hexamethonium, 178
in measurement of cardiac output by direct Fick principle without gas analysis, 161, 165
Para-amino-hippurate vs. nitrous oxide, in renal blood flow measurement, 1180–1183
tubular excretion of, in renal insufficiency, 70, 73, 77
Para-aminohippuric acid
clearance of, in pregnancy, 47
competition of, with diodrast, 1184–1190
Penicillin
and immune response in streptococcal infection, 630–632
in intestinal degradation of choline, 1117–1120
staphylococci resistant to, *582*
for typhoid carriers, *593*
Peptidase
in bile, 678
in serum, 674–679
Peptone, in proteinase production by Cl. histolyticum, 1320–1321
Periarteritis nodosa
blood copper in, 336
serum polysaccharide in, 1210

Peritoneal fluid, agglutination of granulocytes in, 437
Pernicious anemia, 334–336, *598,* 1073
Phagocytosis, and nutritional state, 440
Pharyngitis, streptococcal
antistreptolysin in protection against, *572*
immune response in, 632
Phenobarbital. *See* Barbiturate
Phenol, urinary excretion of, after antibiotics, *598*
Phenylbutazone, and urate clearance
in gout, 605
renal, 1121–1132
Phenylhydrazine, iron absorption in anemia induced by, 1225–1228
Phlebothrombosis, experimental production of, *610*
Phosphatase
acid, of prostatic origin, *575*
alkaline
in blood, 644
in leukocyte, 256
in lymph, 644
serum acid, in prostatic cancer, 1034–1043
Phosphate
inorganic, in muscular dystrophy, 1061–1063
serum inorganic
after fructose infusion, *276, 279*
after glucose infusion, *276, 279*
in urine
after phenylbutazone in gout, 1126
after quiet standing, 245–249
after "6063" in respiratory acidosis, 623, 627
Phospholipid, in liver, *222*
Phosphorus
body pool of, 500–501
in erythrocyte, 499–500, 503, 508, 509
inorganic, after intravenous cinnamate, 716
metabolism of
during administration of corticosterone in rheumatoid arthritis, *772*
during globin administration, 968
in man, 497–509
in plasma, 497–499, 501–503, 508
effect of glucose on, 500
effect of insulin on, 500
in potassium depletion due to laxatives, 265, 266
radioactive
in erythrocyte, 26, 499–500, 503, 508, 509
in plasma, 497–503, 508
urinary excretion of, 500
turnover of, 501
in leukocytes, *553*
in urine, 500
after pitressin, 875
Pigmentation, of skin, spectrophotometric study of, 1053–1059
Pitressin
antidiuresis due to, 289
in liver disease, 422–427
in non-edematous patients, *611*
corticosteroid excretion after administration of, 887–888
and electrolyte excretion, 868–877
sodium diuresis after, *582*
tolerance, in humans, 885–891
Pituitary gland
anterior, and ventral prostate, *609*
follicle-stimulating hormone from, in antihormone formation, 586
posterior, antidiuretic hormone from, 289
Placebo, toxic effects after, *613*
Plasma
dextran as volume expander of, 340–343
globin as expander of, 907
oncotic pressure of, as antidiuresis stimulus, 283–291
pooled, hepatitis after, *601*
proteolytic enzyme in, 473–479
thromboplastin factors in, in hemorrhagic diseases, *599*
thromboplastin inhibitor in, 381–388
volume of, *577*
and body/venous hematocrit ratio, 1309–1316
after hyperoncotic albumin, 283–291
iodinated albumin for measurement of, 107–112
radiochromic chloride for measurement of, 991–995, 1000–1004
T-1824 dye method for measurement of, 107–112
tagged erythrocyte for measurement of, 111
Plasmapheresis, regeneration of plasma proteins during, *550*
Plasmin, substrates for, 473–479
Plastic, ball valve made of, for severe aortic insufficiency, *598*
Platelet
antigenicity of, *606*
concentration of, and fibrin strand width, *571*
grouping of, *606*
Pleocytosis, after streptokinase-streptodornase in subarachnoid space, 392
Pleura, biopsy of, *597*
Pneumococcus
capsular polysaccharides of, as antigen in leukemia, 317–320
pneumonia due to
erythromycin for, *557*
magnamycin for, *557*
Pneumonia
blood copper in, 332
pneumococcal
erythromycin for, *557*
magnamycin for, *557*
Poiseuille's law, in determination of blood viscosity, 292–297
Polarography, in study of oxygen tension of tissues, 1097–1099
Poliomyelitis, viremia in, *576*
Polycythemia
arterial blood in, 52–58
blood copper in, 336
of congenital heart disease, 56, 57
erythrocyte ultrastructures in, 983
hemoglobin in, 57
leukocyte alkaline phosphatase in, 256
with leukocytosis, leukocyte glycogen in, 255
lung in, 57
methemoglobin in, 56
myeloid leukocyte glycogen in, 255, 256
pulmonary alveolus in, 57
Polysaccharide
granulocyte movement toward, 438–442
non-glucosamine, bound to serum protein, 416
pneumococcus, as antigen in leukemia, 317–320
in serum
in diabetes mellitus, 415–419
in rheumatoid arthritis vs. degenerative joint disease, 1208–1213
Portal vein
effect of portacaval shunt on hepatic blood flow, 526–536
pressure in, after hexamethonium, 796, 799
Position (of patient)
and effect of hexamethonium on renal hemodynamics, 1134, 1138
and intracranial cerebrospinal fluid pressure, 848
and lobar alveolar gas concentration, 617–621
and oxygen tension of digital skin, 1097–1099
and protein excretion, *582*
and pulmonary tuberculosis, 620
and pulsus alternans, *562*
release of catechol amines in hypotension due to, 1065–1069
and solute diuresis, 242–249
and water excretion, *594*
and water loading related to solute excretion, 398
Posture. *See* Position
Potassium
in blood, after "6063" in respiratory acidosis, 623, 627
and carbohydrate metabolism, 19–21
depletion of, *605*
due to laxatives, 258–270
dietary deficiency of, in hypertension, 633–636
exchange of, in sickle cell anemia red cells, *608*
excretion of
after enhanced sodium reabsorption, *613*
after hexamethonium, 178, 182–183
effects of magnesium sulfate on, 858–861
after oral hexamethonium, 1136–1137, 1138
in renal insufficiency, 69–71
after vena caval congestion, 1150, 1155, 1156, 1158
metabolism of
during administration of corticosterone in rheumatoid arthritis, *772*
by liver mitochondria, *591*

Potassium (*Cont'd*)
in nephrectomized rats, with hypokalemic alkalosis, 538–541
in plasma, in diabetic acidosis, 114–116
in prevention of ACTH-induced sodium retention, 1197–1207
radioactive, in diabetes mellitus, 15–21
renal excretion of
and arteriovenous fistula, 237
and diuresis after water loading, 396, 398–400
and life situations, *602*
vs. rubidium, in acid-base balance, *597*
in serum, in diabetic ketosis, 1215
in solution in colon, 447
in sweat, 739–740, 742–744
transfer of, in adrenal insufficiency, 1240, 1244
in urine
in diabetic ketosis, 1215
after hyperoncotic albumin, 283–291
after phenylbutazone in gout, 1126
after "6063" in respiratory acidosis, 623, 626–628
after water diuresis in liver disease, 425
Potassium chloride, and water loading, related to solute excretion, 399, 400
Potassium oxalate, and "L. E. cell" phenomenon, 199
Pre-eclampsia, renal function in, 44–50
Pregnancy
apresoline in, 922–929
blood copper in, 329, 331
electrolyte excretion in, 46, 49
measurement of uterine blood flow in, *589*
renal function in, 44–50
toxemia of, 44–50
Pressure
of blood. *See* Blood, pressure of
fistula, 234
effect of, on stored blood, 1007–1011
intracardiac, in patients with arteriovenous fistula, 543–546
left atrial, in mitral stenosis, *554*
pulmonary, in mitral stenosis, 523
pulmonary arterial, in emphysema, 131–135
pulmonary capillary
in emphysema, 132–135
in mitral stenosis, *554*
in mitral valvular disease, 519–521
Pressure pulse wave, in mitral stenosis, *554*
Priscoline, cerebral effects of, 125–128
Probenecid, and phenylbutazone in gout, 1128
Proconvertin, 915–920
Prostate
cancer of, serum acid phosphatase in, 1034–1043
serum acid phosphatase from, *575*
ventral, effect of anterior pituitary on, *609*
Prosthesis, plastic valvular, for severe aortic insufficiency, *598*
Protease, in plasma, 473–479
Protein
bacterial digestion of, 1317–1322, 1323–1329
dietary
and blood pressure, 189, 190
and weight loss, 185–191
globin as food supplement, 964–971
globin as source of, 900–907
iodine bound to, in thyroid disease, 149–159
M, of group A streptococci, 1085–1092
in plasma
exchange of, with ascitic fluid, *553*
during plasmapheresis, *550*
regeneration of, *550*
tagged with radioactive chromate, *571*
in serum, 1215
cationic detergent assay of, 661–672
electrophoresis in assay of, 667–669
after globin administration, 965
in leukemia, *600*
in malignant lymphoma, *600*
non-glucosamine polysaccharides bound to, 416
in rheumatoid arthritis vs. degenerative joint disease, 1211
in tissue, and in vivo reactions with allergen, *564*
in urine
during globin administration, 967–968
in renal disease, *582*
Proteinase
bacterial, 1317–1322, 1323–1329
from Cl. hystolyticum, 1323–1329
Proteinate, in serum, in cyanotic congenital heart disease, 839
Proteolysis
due to enzyme in plasma, 473–479
due to thrombin, *603*
Proteus vulgaris, in digestion of collagen, 1318–1319
Prothrombin, 915–920
Protoveratrine
intravenous, *561*
and renal function, 80–88
Pseudoglobulin, in serum, in rheumatoid arthritis vs. degenerative joint disease, 1211, 1212, 1213
Pseudohemophilia, dual hemostatic defect in, *551*
Psychosis
after ACTH, 372, 379
and cerebral blood flow, 463
Pulmonary artery
pressure in
in emphysema, 131–135
after hexamethonium, 1286
in patients with arteriovenous fistula, 544, 546
Pulse, and arteriovenous fistula, 234
Pulse pressure
pulmonary capillary, in mitral stenosis, *554*
renal arterial, 238
Pulse rate, after venous congestion of limb, 1111
Pulse-volume digital, after intravenous protoveratrine, *561*
Pulsus alternans, orthostatic factors in, *562*
Pyrodine. *See* Acetylphenylhydrazine
Pyruvic acid
after fructose infusion, 277–278, 279–280
after glucose infusion, 277–278, 279–280

Q

Q-T interval, in Laennec's cirrhosis, 1030

R

Rabbit
Rickettsia mooseri toxin in, *594*
streptococcus-infected, fibrinoid necrosis of coronary artery in, *562*
Radiocarbon, in glycine, 1–4, *554*
Radiochromium
as biological tracer, *571*
in determination of erythrocyte mass, 1000–1004
in determination of erythrocyte survival, 1260–1276
in determination of plasma volume, 991–995, 1000–1004
Radioiodine
albumin tagged with, 29, *601*
metabolism of, 746–767
in blood flow in bone marrow, 952–962
globin tagged with as blood volume expander, 22–31
in human serum albumin, 138–145
for measurement of plasma volume, 107–112
salivary concentration gradients for, 1077–1083
and thyroid stimulating hormone, 149–159
and thyroidal function, *577*
thyroxine, labeled with, *596*
triiodothyronine labeled with, *596*
urinary excretion of, in thyroid disease, 153
Radiophosphorus
in erythrocyte, 26, 499–500, 503, 508, 509
in plasma, 497–503, 508
urinary excretion of, 500
Radiopotassium, in diabetes mellitis, 15–21
Radiosodium
and extracellular fluid volume, 483–488
in renal insufficiency, 75, 76
Radiosulfate
in measurement of extracellular fluid, 299–309
in hypertension, 312–316
Rapid slide agglutination method for Brucella agglutinin, 98–99, 104

Rat
adrenalectomized, cortisone acetate and desoxycorticosterone acetate in, 1175–1179
nephrectomized, with hypokalemic alkalosis, 538–541
Rickettsia mooseri toxin in, *594*
Recticulocytosis
in sickle cell anemia, 1074
in sickle cell trait, 1075
Red blood cell. *See* Erythrocyte
Reflex, vasoconstrictor, after hexamethonium, 1295
Resistance
cerebral vascular, 33–37
cerebral vascular after priscoline, 127
glomerular arteriolar, after protoveratrine, 87
pulmonary, in emphysema, 133
renal vascular, in pregnancy, 47–48, 50
Respiration
bacterial, after streptomycin, *593*
in Boeck's sarcoid, 909–912
and effect of carbon dioxide tension on bicarbonate reabsorption, 975–977
and cardiac output, 6–7
at high altitude, 490–496
in mitral valvular disease, 510–524
Respirator, and respiratory gas exchange, in pulmonary emphysema, 1192–1196
Respiratory disease, due to virus, *552*
Respiratory gas exchange, effect of mechanical exsufflation on, 1192–1196
Rest, cardiac output during, in Laennec's cirrhosis, 1025–1032
Resting minute ventilation and gas exchange, at high altitude, 491, 494–495
Rheumatic disease, effect of corticotropin on body water and electrolytes in, 879–883
Rheumatic fever
vs. glomerulonephritis, 362
prevention of cardiac abnormalities due to, *572*
immune response in streptococcal infections, 630–632
and serological type of streptococcus, 352–353
susceptibility to, as related to antibody response to non-streptococcal antigen, 691–695
Rheumatism, myocardial insufficiency in mitral stenosis due to, *566*
Ribonuclease, in inhibition of desoxyribonuclease, 195
Rubidium, vs. potassium, in acid-base balance, *597*

S

"6063," in respiratory acidosis, 622–628
Saccharide, and leukocyte movement, 436–442
Saline solution, 34
change in ionic composition of, in colon, 444–448
hypertonic
in diabetes insipidus, *607*
after gastrectomy, *597*
pain due to, 1045–1051
intravenous, and cerebral blood flow, 34
isotonic diuretic response to, 807–811
Saliva
concentration gradients for iodide and chloride in, 1077–1083
electrolyte in, *569*
electrophoretic pattern of, 863
Salt
acidifying, after sodium depletion, *602*
in congestive heart failure, *579*
dietary
and blood pressure, 188–189
and inulin space in adrenal insufficiency, 1242
excretion of
and arteriovenous fistula, 235–239
after intracranial pressure-volume change, 848–849
and posture, *594*
after venous congestion of limbs, 1101–1115
and reaction of Octab with protein, 664–665
renal excretion of, and diuresis after water loading, 396–402
solution of, for dehydration in diabetic acidosis, 114
urinary excretion of, after hyperoncotic albumin, 283–291
Sarcoid, Boeck's, pulmonary function in, 909–912
Sarcoidosis, of liver, cationic detergent assay of serum protein in, 664
Saturation, arterial, in polycythemia, 54, 56–58
Scarlet fever
acute glomerulonephritis after, 346–348
antistreptolysin in protection against, *572*
Sclerosis, vascular, and cerebral blood flow, 459–465
Screening test, Castaneda, for Brucella agglutinin, 99–100, 104
Sedimentation rate, in rheumatoid arthritis and degenerative joint disease, 1212
Serum
complement
as indication of inflammation, *568*
in nephrotic syndrome, *581*
proteolytic enzyme in, 477
thromboplastin inhibitors in, 382
vitamin B_{12} binding capacity of, *595*
Serum acid phosphatase, in prostatic cancer, 1034–1043
Sex (of patient), and exchangeable potassium content, 20
Shock, hemorrhagic, blood flow and transcapillary exchange in, *612*
Shunt, portacaval, and hepatic blood flow, 526–536
Sickle cell anemia
filter paper electrophoresis of hemoglobin in, 1073, 1074
potassium in erythrocyte in, *608*
Sickle cell-Hemoglobin C disease, 1282–1283
Sickle cell trait, filter paper electrophoresis of hemoglobin in, 1074
Skin
blood copper in pyogenic abscess of, 332
erythematous response of, to ultraviolet light, 681–689
measurement of extracellular phase of, 1299–1308
temperature of
after apresoline in pregnancy, 925, 929
after intravenous protoveratrine, *561*
ultraviolet erythema and pigmentation of, 1053–1059
Slide test, Castaneda, for Brucella agglutinin, 99–100, 104
Sodium
and arteriovenous fistula, 235–239
in blood
after pitressin, 886–887, 889–891
after "6063" in respiratory acidosis, 623, 627
effect of corticotropin on, in rheumatic disease, 879–883
depletion of, acidifying salts after, *602*
dietary, and blood pressure, 188–189
distribution of, in disease, *612*
excretion of
after hexamethonium, 177–178, 181–183
after intracranial pressure-volume change, 848–849
effects of magnesium sulfate on, 858–861
after oral hexamethonium, 1135–1138
after pitressin, *582*
after vena caval congestion, 1150, 1155, 1156, 1157, 1158
after venous congestion of limbs, 1101–1115
intracellular, in diabetic acidosis, 118
metabolism of
during administration of corticosterone in rheumatoid arthritis, 772
after pitressin, *582*, 885–887
in muscle, in hypokalemic alkalotic rat, 539
in plasma
in hypokalemic alkalotic rat, 539
after phenylbutazone, 1127
after pitressin, 886–887, 889–891
in potassium depletion due to laxatives, 264, 265, 266
potassium in prevention of ACTH-induced retention of, 1197–1207

Sodium (*Cont'd*)
radioactive,
and extracellular fluid volume, 483–488
in renal insufficiency, 75, 76
renal excretion of
and diuresis after water loading, 396–402
and life situations, *602*
in renal insufficiency, 68–78
renal tubular reabsorption of
after Compound E, *613*
after Compound F, *613*
after DOCA, *613*
retention of
in cirrhosis of liver, *560*
in heart disease, *566*
in serum
in adrenalectomized rat, 1177–1178
in diabetic ketosis, 1215
after hepatic and cardiac disease, 934, 937
in solution in colon, 444–448
space, 483–488
in sweat, 738–739, 742–744
transfer of, in adrenal insufficiency, 1238–1241
in congestive heart failure, *579*
in diabetic ketosis, 1216
after hyperoncotic albumin, 283–291
after leg tourniquet, 1167
after phenylbutazone in gout, 1126, 1131
after pitressin, 868–877
after pitressin in non-edematous patients, *611*
in pregnancy, 46, 49
after quiet standing, 242–249
after "6063" in respiratory acidosis, 623, 626–628
after water diuresis in liver disease, 425
urinary corticoid in retention of, *585*
See also Electrolyte
Sodium acetate, and ketone body production, *584*
Sodium amytal. *See* Barbiturate
Sodium arsenate, and "L. E. cell" phenomenon, 199
Sodium bicarbonate
and cerebral circulation, 34–37
and water loading related to solute excretion, 399, 400
Sodium chloride
and "L. E. cell" phenomenon, 199
and reaction of Octab with protein, 664–665
solution of, in colon, 444–448
Sodium chromate, radioactive, in determination of red cell mass and plasma volume, 1000–1004
Sodium cinnamate, for evaluation of liver function, 711–718
Sodium citrate, and "L. E. cell" phenomenon, 199
Sodium octanoate, intravenous, splanchnic ketone production after, *610*
Solute
excretion of, in edema, *570*
renal excretion of, and diuresis after water loading, 394–402
urinary excretion of
after hyperoncotic albumin, 283–291
after quiet standing, 242–249
Sound, Korotkoff, *598*
Space
antipyrine, 75
dead, in mitral valvular disease, 517–518
inulin, 75–76
sodium, 483–488
sucrose, 483–488
T-1824 dye, 1013–1019
thiosulfate, 1299–1308
Species specificity, in inhibition of, desoxyribonuclease, 196–197, 199–200
Spectrophotometry, in study of ultraviolet erythema and pigmentation, 1053–1059
Spirography, for evaluation of pulmonary function prior to surgery, *571*
Splanchnic area
blood flow in, after hexamethonium bromide, 793–799
oxygen consumption of, during hypotension due to bleeding, *573*
Splanchnicectomy
and venous congestion of limbs upon renal function, 1101–1115
renal electrolyte excretion after, *589*
Splenectomy, and thalassemia major with associated acquired hemolytic anemia, 1233–1235
Splenomegaly, and body/venous hematocrit ratio, 1315
Spondylitis, rheumatoid, serum polysaccharide in, 1210
Spore-bearer, aerobic, in digestion of collagen, 1318
Standing, quiet, solute diuresis after, 242–249
Staphylococcus
in digestion of collagen, 1318
resistant to antibiotic, *582*
Starch, granulocyte movement toward, 438–442
Stenosis, mitral. *See* Mitral valve, stenosis of
Steroid
adrenal
direct estimation of, 703–709
after pitressin, 888–889
in stress, *608*
adrenal cortical, intravenous, *579*
metabolism of, *573*, 950–951
alpha-ketosteroid patterns, 940–948
reproducibility of patterns of, 950–951
Stilbestrol, and serum acid phosphatase in prostatic cancer, 1041
Stomach, acidity, in absorption of iron from upper gastrointestinal tract, *571*
Stools, carbon-14 excretion in, 2
Streptococcus
antistreptolysin in, immunity against, *572*
fibrinoid necrosis of coronary artery in rabbits infected with, *562*
glomerulonephritis after infection due to, *606*
immune response after infection with, 630–632
serological type of
and acute glomerulonephritis, 348–355, 359–363
and rheumatic fever, 352–353
type specific antibody against, demonstrated by hemoglobin technique with tannic acid, 1085–1092
Streptodornase, *579*
subarachnoid injection of, 391–393
Streptokinase
in activation of proteolytic enzyme in plasma, 473–479
subarachnoid injection of, 391–393
Streptomycin, antibacterial action of, *593*
Stress
and adrenal cortex
in carbohydrate metabolism, 781–790
reserve, *608*
and eosinophils, *581*
Stroma, of erythrocyte, 1251
Stupor, episodic, due to Eck fistula, *587*
Subarachnoid space, streptokinase-streptodornase in, 391–393
Sucrose space, 483–488
Sugar
in blood, in diabetes mellitus, *577*
and leukocyte metabolism, 1171–1174
in urine, in diabetes mellitus, *577*
Sulfaphthalidine in intestinal degradation of choline, 1117–1120
Sulfate, radioactive, for measurement of extracellular fluid, 299–309, 312–316
Surgery, and serum acid phosphatase in prostatic cancer, 1039
Sweat
electrolyte in, 736–744
nitrogen in, 740–744
Sweat glands, urea clearance by, *602*
Sympathectomy
epinephrine hypersensitivity after, 851–856
thoraco-lumbar, and protoveratrine, 86
Sympathetic nervous system and renal function, 182
Synovial cavity, metabolism of hydrocortisone in, *614*
Synovial fluid, after intra-articular hydrocortisone, 480–482

T

T-1824 dye
in human serum albumin, 138–145
in localization of cardiac defects by dilution curves, *613*
for measurement of plasma volume, 107–112

space, in diarrhea of infancy, 1013–1019
Tannic acid, in demonstration of type specific streptococcal antibody, 1085–1092
Tartrate, and serum acid phosphatase in prostatic cancer, 1034–1043
Temperature
effect of, on stored blood, 1007–1011
of skin
after apresoline in pregnancy, 925, 929
after intravenous protoveratrine, *561*
Tendon, measurement of extracellular phase of, 1299–1308
Terramycin, in intestinal degradation of choline, 1117–1120
Test
for Brucella agglutinin in serum, 96–105
cold-pressor, cardiac output during, *559*
hemolytic, for tuberculosis, *590*
Thalassemia major
associated with acquired hemolytic anemia, effect of splenectomy on, 1233–1235
extracorpuscular defect in, 1229–1233
Thiocyanate
and response of thyroid gland to thyrotrophin, 156–157
and salivary transport mechanisms, 1082–1083
Thiosulfate, space, in measurement of extracellular fluid, 1299–1308
Thoracic duct, characteristics of lymph from, 637–649
Thrombin
concentration of, and fibrin strand width, *571*
formation of, 917–920
"lag phase," 920
proteolytic action of, *603*
Thromboembolism, anticoagulants for, *652*
Thromboplastin
action of, 918–919
inhibitor of, 381–388
plasma factors of, in hemorrhagic diseases, *599*
Thrombosis
anticoagulants for, *652*
cerebral, carbon dioxide inhalation for, 700–701
intravascular, experimental production of, *610*
Thyroid gland
function of, after injury of thyrotropin, *583*
hormonal stimulation of, and radioiodine metabolism, 149–159
hormone from, 149–159
Thyroid hormone
exchangeable pool of, 453–457
synthesis of, *577*
utilization of, *558*
Thyrotrophin
and radioiodine metabolism, 149–159
thyroid function after injection of, *583*
Thyroxine
exchangeable pool of, 453–457
labelled, metabolism of, *596*
in myxedema, *552*
radioactive, metabolism of, in rat, *580*
Tidal volume, in mitral valvular disease, 517–518
Tissue
breakdown of, and potassium loss in diabetes mellitis, 19–20
connective, in maintenance of extracellular fluid composition, *583*
electrolyte in
in DCA hypertension, *608*
in diabetic acidosis, *580*
iodo-albumin removal from, 755
measurement of extracellular phase of, 1299–1308
osmotic equilibria of, *556*
protein in, and in vivo reactions with allergen, *564*
supportive, pain due to hypertonic saline in, 1045–1051
Toe, effect of position on oxygen tension of skin of, 1097–1099
Tourniquet, and renal function, 1163–1169
Toxemia of pregnancy
apresoline in, 924–927
renal function in, 44–50
Toxicity, after placebo, *613*
Toxin, rickettsial, *594*
Trait, sickle cell, filter paper electrophoresis of hemoglobin in, 1074
Transfusion
erythrocyte survival after, *576*
metabolism of heavy nitrogen labelled erythrocytes after, *578*
Treponema pallidium, and adhesion-disappearance phenomenon, *592*
Triiodothyronine
l-, in myxedema, *552*
labelled, metabolism of, *580, 596*
Trimethylamine, formation of, after ingestion of antibiotics and choline, 1117–1120
Tripeptidase
in bile, 678
in serum, 674–679
Trypsin, in inhibition of desoxyribonuclease, 195
Tube dilution agglutination method, for Brucella agglutinin, 98–99, 103–104
Tubercle bacillus, in vivo behavior of, *587*
Tuberculosis
blood copper in, 332
hemolytic test for, *590*
pulmonary, effect of body position on, 620
Tubule, renal
in hypokalemic alkalosis, 540–541
and PAH excretion, 178
in renal insufficiency, 70, 73, 77
Turbidimetry, using cationic detergents, in assay of serum proteins, 661–672
Typhoid fever
antibody response of rheumatics to immunization against, 692–694
blood copper in, 332
treatment for carriers of, *593*

U

Ulcer, duodenal, electrophoretic patterns in gastric juice of, 863, 865
Ultraviolet light, erythematous response of human skin to, 681–689, 1053–1059
Unconsciousness and cerebral oxygen, consumption, 464
Unsaturation, arterial, in polycythemia vera, 54, 57, 58
Urate, clearance of
after phenylbutazone, *605,* 1121–1132
after probenecid, 1128–1130
Urea
clearance of, by sweat glands, *602*
renal excretion of, and diuresis after water loading, 396–402
urinary excretion of after hyperoncotic albumin, 283–291
Urea nitrogen
blood, during globin administration, 966–967
serum, in adrenal insufficiency, 1238, 1241
Uremia, serum inulinoid blank in, 212
Uremic syndrome, acid-base balance during hemodialysis for, 729–735
Ureteroenterostomy, acidosis after, 447
Uric acid
glycine nitrogen for synthesis of, in gout, 775–777
nitrogen for synthesis of, in gout, 778–780
Urine
after hexamethonium, 176–178, 180–183, 1135–1137, 1293
after hyperoncotic albumin, 283–291
after leg tourniquet, 1167
after protoveratrine, 86–87
during vena caval congestion, 1150, 1155, 1158, 1159
after venous congestion of limbs, 1101–1115
after water diuresis in liver disease, 424
See also Diuresis
Urobilinogen, dextrorotatory, crystalline, *585*
Uterus, blood flow in, in pregnancy, *589*

V

Valsalva maneuver, circulatory phenomena produced by, *588*
Valve
aortic, plastic valvular prosthesis for insufficiency of, *598*
mitral. *See* Mitral valve

Vascular system
cerebral
after priscoline, 125–128
resistance of, 33–37
renal, in pregnancy, 47–48, 50
stretching of, as stimulus for electrolyte and water excretion, 1159–1160
Vasoconstriction
cerebral
due to l-nor-epinephrine, 230
after priscoline, 127
Vasoconstrictor reflex, after hexamethonium, 1295
Vasodilation
cerebral, after priscoline, 127
renal, after protoveratrine, 87–88
Vasopressor reflex, after apresoline in pregnancy, 926, 928
Vein
congestion of, in limbs, and renal function, 1101–1115
stretching of, as stimulus for electrolyte and water excretion, 1159–1160
Vena cava
intracardiac catheter, for producing increased pressure in, 1140–1144
effect of portacaval shunt on hepatic blood flow, 526–536
renal effects of congestion of, 1145–1161
Venipuncture, antidiuresis after, 289
Ventilation
and effect of position on lobar alveolar gas concentration, 620
in mitral valvular disease, 510–524
pulmonary
in emphysema, *570*
at high altitude, 490–496
Ventricle, right, pressure in
after hexamethonium, 1286
in patients with arteriovenous fistula, 544–546
Veratrum. *See* Protoveratrine
Viobin, 60
Viremia, in poliomyelitis, *576*
Virus
hepatitis due to, serum cholinesterase in, 466–471
respiratory disease due to, *552*
Viscera, oxygen consumption of, during hypotension due to bleeding, *573*
Vital capacity, in mitral valvular disease, 515–518, 521
Vitamin B_{12}, binding capacity of human serum for, *595*
Volume, tidal, in mitral valvular disease, 517–518

W

Water
effect of adrenal cortex on distribution of, 1236–1247
effect of corticotropin on, in rheumatic disease, 879–883
in diabetic ketosis, 1216
diuresis due to
in liver disease, 422–427
related to renal excretion of solutes, 394–402
in erythrocyte, in diabetic acidosis, 118–119
excretion of
in congestive heart failure, *579*
after enhanced sodium reabsorption, *613*
after hyperoncotic albumin, 283–291
after oral hexamethonium, 1135–1138
and posture, *594*
in pregnancy, 45–46
during renal insufficiency, 69, 76–77
after venous congestion of limbs, 1101–1115
effect of exogenous adrenal steroid on, after pitressin, 888–889
extracellular
measurement of, 1299–1308
volume of, 75, 77
ingested, after pitressin, *582*
intravenous, for diuresis in liver disease, 422–427
metabolism of, after pitressin, 885–887
in plasma, in diabetic acidosis, 114
redistribution of, in adrenal cortical insufficiency, *574*
renal excretion of, and life situations, *602*
in renal insufficiency, 75–77
retention of
after antidiuretic hormone in hepatic and cardiac disease, 931–938
and electrolyte excretion, 868–877
in heart disease, *566*
after pitressin in non-edematous patients, *611*
transcellular, 308
See also Body fluid; Fluid
Wave length, and erythematous response of human skin, 685–689
Weber Christian Disease, serum polysaccharide in, 1210
Weight of body. *See* Body weight
White blood cell
extract of, in inhibition of desoxyribonuclease, 193–197, 199–200
glycogen in, 251–256
effect of saccharides on movement of, 436–442
susceptibility of, to "L. E. cell" transformation, 197
See also Leukocyte
Wilson's disease, blood copper in, 336

X

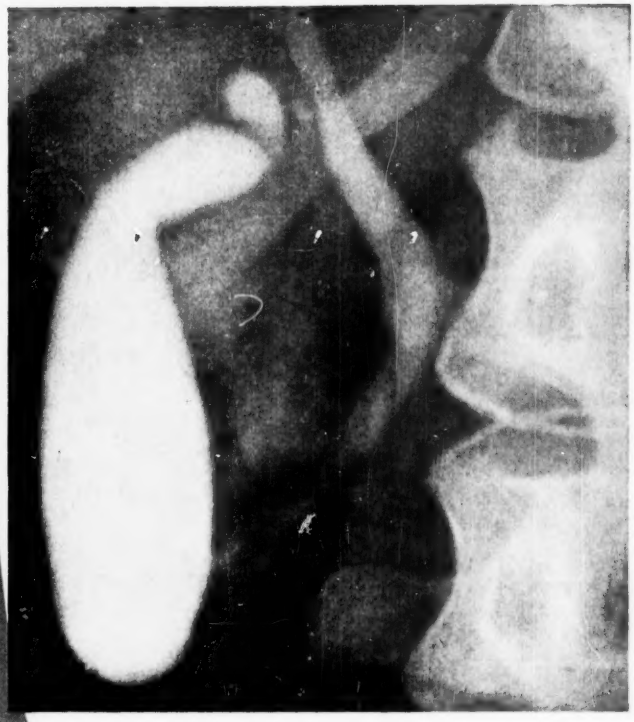
Xanthomatosis, hypercholesterolemic, heredity of, *611*

Y

Yeast, desoxyribonuclease inhibitor in, 199
Yeast protease, in inhibition of desoxyribonuclease, 195

WINTHROP

"...the bile ducts are visualized in their natural and 'undisturbed' state..."[1]



BILE DUCT VISUALIZATION WITH

Telepaque®

Superior Oral Cholecystographic AND CHOLANGIOGRAPHIC Medium

The frequency of bile duct visualization with Telepaque plus the high incidence of dense gallbladder shadow[2] are advantages of distinct diagnostic importance. Furthermore, Telepaque is notable for its low degree and percentage of side reactions.

DOSAGE: The average adult dose of Telepaque is 6 tablets with at least one glass of water from ten to twelve hours before the scheduled roentgen examination.

SUPPLIED in tablets of 0.5 Gm., envelopes of 6 tablets, boxes of 5 and 25 envelopes; bottles of 500 tablets.

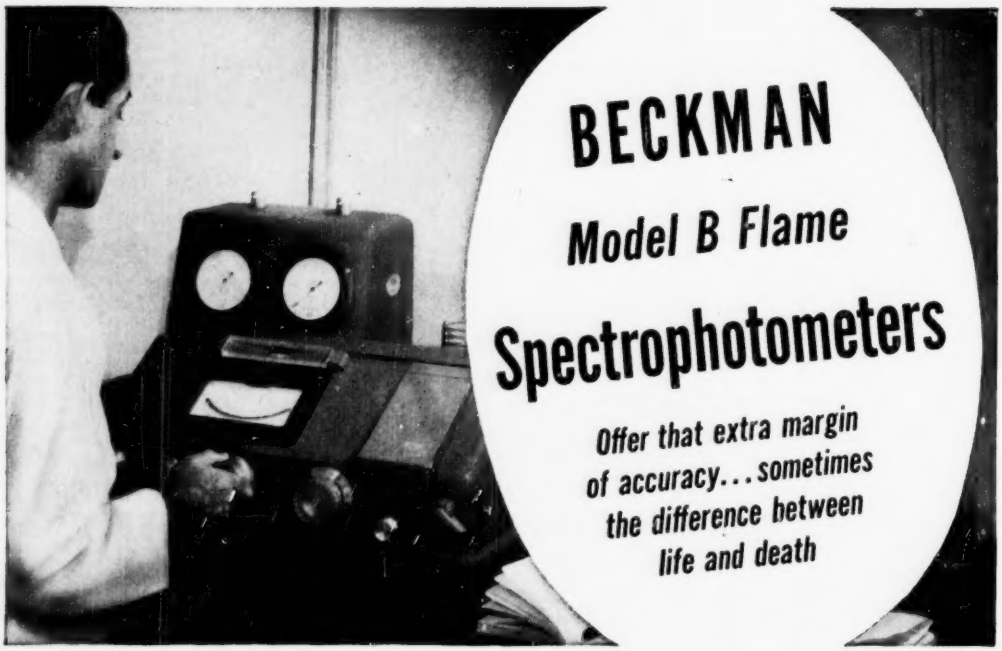
1. Steinbach, W. H.: Am. Jour. Roentgenol., 68:160, Sept., 1952.

2. Everett, E. F., and Rigler, L. G.: Radiology, 58:524, Apr. 1952.

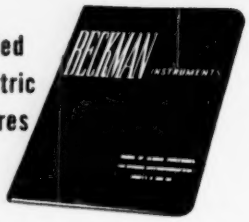
Telepaque, trademark reg. U. S. & Canada, brand of iodopanoic acid

WINTHROP-STEARNS INC. • **New York 18, N. Y.** • **Windsor, Ont.**

"A COMPLETE ANALYTICAL LABORATORY *in ONE PACKAGE"*


BECKMAN
Model B Flame
Spectrophotometers
Offer that extra margin of accuracy... sometimes the difference between life and death

Simplified Colorimetric Procedures



This new handbook details 13 laboratory-proven procedures for making step-by-step analyses with Beckman Spectrophotometers. It is authoritative, will save much time in your lab and costs only $5.00 through your Beckman dealer.

A prominent doctor recently reported that the extreme accuracy of the Beckman Model B permitted detection of a critical potassium deficiency and subsequent corrective therapy which saved the patient's life. Top authorities state that, because of inadequate instrumentation, many clinical analyses being made today are unreliable and do not offer a sound basis for diagnosis and therapy.

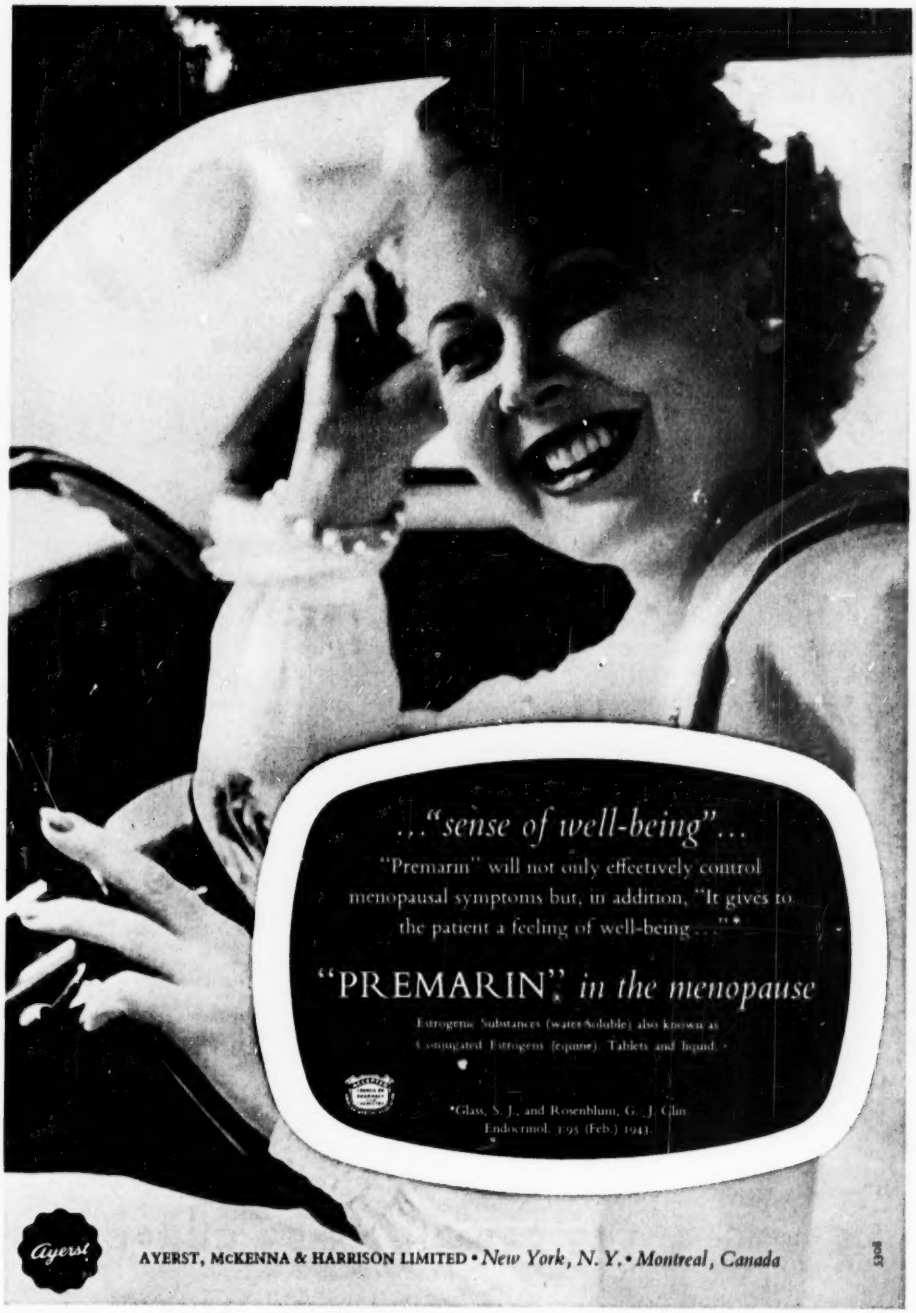
Only the Beckman Model B provides the full accuracy available in a modern spectrophotometer with complete ease of operation.

- ✓ Fastest and most accurate for sodium, potassium and calcium in blood, serum and plasma... best for ALL clinical flame analyses.
- ✓ Better and more practical for colorimetric analyses than any colorimeter ...a complete analytical lab in one package!

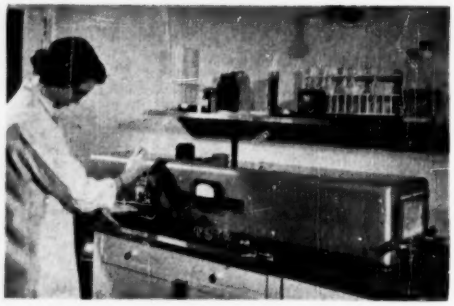
See your Beckman dealer or write for Data File 26-316.

Beckman *division*

BECKMAN INSTRUMENTS, INC.
SOUTH PASADENA 1, CALIFORNIA


...“sense of well-being”...
“Premarin” will not only effectively control menopausal symptoms but, in addition, “It gives to the patient a feeling of well-being...”*
“PREMARIN” in the menopause
Estrogenic Substances (water-soluble) also known as Conjugated Estrogens (equine). Tablets and liquid.
*Glass, S. J., and Rosenblum, G. J. Clin. Endocrinol. 3:95 (Feb.) 1943.
Ayerst
AYERST, McKENNA & HARRISON LIMITED • New York, N. Y. • Montreal, Canada
308

The Portable, Complete Electrophoresis Apparatus



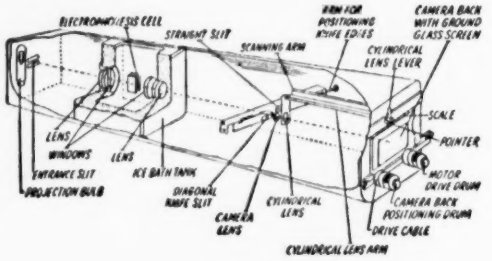
Perkin-Elmer Tiselius Electrophoresis Apparatus at the University Hospital of the New York University—Bellevue Medical Center.

An ordinary laboratory bench, without special mountings, is sufficient for the installation of The Perkin-Elmer Tiselius Electrophoresis Apparatus. Precision-ground, achromatic schlieren optics yield sharp, clear patterns. Operating the instrument is so simple that highly trained personnel are not required—a single technician can readily operate two units. Measuring only 63 inches in length, the entire unit weighs 120 pounds and is constructed of heavy aluminum. It operates for several hundred hours under its own power system.

The apparatus is shipped complete and prefocussed with a 2 cc Tiselius cell 50 mm long, cell holder, ice bath and stirrer, silver-silver chloride electrodes, open-end 250 cc buffer bottles, stainless steel needles for filling the cell, ground glass screen for visual observation of the pattern, and a 3¼" x 4¼" film holder. Double knife-edge and diagonal slit for use with a cylindrical lens are also included.

The dependability, accuracy and simplicity of The Perkin-Elmer Tiselius Electrophoresis Apparatus have made it the choice of hospital and research laboratories throughout the world.

Write today for complete information.



Schematic cutaway of the new Perkin-Elmer Model 38 Tiselius Electrophoresis Apparatus.

THE PERKIN-ELMER CORPORATION

Norwalk, Connecticut

Leading manufacturers of Infrared Spectrometers, Continuous Infrared Analyzer, Universal Monochromator, Flame Photometer, Tiselius Electrophoresis Apparatus, D. C. Amplifiers, and other electro-optical instruments for analysis and research.

1 Just load dirty pipettes in the basket as they become soiled. Basket rests in cleansing solution.
2 When basket is full, transfer to washer. Three minutes will flush residues away completely, leaving pipettes sparkling clean.
3 Remove basket and put in drying oven.
4 Take out basket and use as dispenser for clean pipettes.

have lots of clean pipettes always on hand *without drudgery*

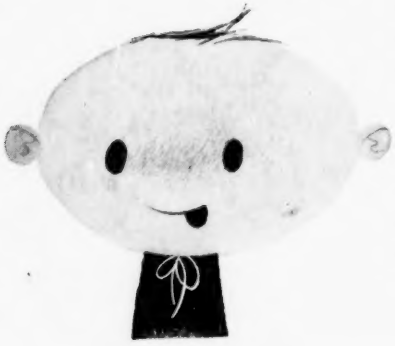
As you can see above, it's no problem at all to have plenty of clean pipettes always available — when you entrust the job to the Technicon Automatic Pipette Washer. Twice every minute the washer swirls seven surging gallons of water through the pipettes inside, flushing away clinging residues. In three minutes (six syphon-cycles) it delivers a full basket-load of immaculate pipettes (holds up to 200 pipettes 1 ml and up to 18" long). The special alloy stainless steel basket is proof against corrosive reagents, even potassium dichromate and sulfuric acid. It carries pipettes as a unit-load through cleansing, washing, drying. Requiring no handling of separate pipettes, it practically eliminates breakage and chipping. Bulletin #6150 gives particulars — let us send it to you.

technicon
automatic pipette washer

THE TECHNICON COMPANY, 215 East 149 Street, New York 51, N. Y.

Taste Toppers . . . for all ages

that's what physicians and patients alike call these two favorite dosage forms of Terramycin because of their unsurpassed good taste. They're nonalcoholic — a treat for patients of all ages, with their pleasant raspberry taste. And they're often the dosage forms of first choice for infants, children and adults of all ages.



Terramycin®

BRAND OF OXYTETRACYCLINE

Pediatric Drops

Each cc. contains 100 mg. of pure crystalline Terramycin. Supplied in 10 cc. bottles with special dropper calibrated at 25 mg. and 50 mg. May be administered directly or mixed with nonacidulated foods and liquids. Economical 1.0 gram size often provides the *total dose* required for treatment of infections of average severity in infants.

Supplied: Bottles of 1.0 Gm.

Oral Suspension *(Flavored)*

Each 5 cc. teaspoonful contains 250 mg. of pure crystalline Terramycin. Effective against gram-positive and gram-negative bacteria, including the important coli-aerogenes group, rickettsiae, certain large viruses and protozoa.

Supplied: Bottles of 1.5 Gm.

Pfizer **PFIZER LABORATORIES,** *Brooklyn 6, N. Y., Division, Chas. Pfizer & Co., Inc.*

LANCASTER PRESS, INC., LANCASTER, PA.